From Departments of Occupational Medicine and Environmental Hygiene.
Lund University, Sweden

METABOLISM OF INORGANIC LEAD AT OCCUPATIONAL EXPOSURE

by

Andrejs Schütz

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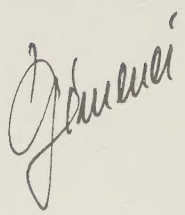

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This thesis is based on the following papers, referred to by their Roman numerals:

I. Schütz, A. and Skerfving, S., 1976, Effect of a short, heavy exposure to lead dust upon blood lead level, erythrocyte delta-aminolevulinic acid dehydratase activity and urinary excretion of lead, delta-aminolevulinic acid, and coproporphyrine, Scand. J. Work Environ Hlth 3:176-84.

II Schütz, A., Skerfving, S., Ranstam, J., and Christoffersson, J.O., Kinetics of lead in blood after end of occupational exposure. Submitted for publication.

III. Christoffersson, J.O., Schütz, A., Ahlgren, L., Haeger-Aronsen, B., Mattsson, S., and Skerfving, S., 1984, Lead in finger-bone analysed *in vivo* in active and retired lead workers, Am. J. Ind. Med. 6:447-457.

IV. Christoffersson, J.O., Schütz, A., Skerfving, S., Ahlgren, L., and Mattson, S., In press, Decrease of skeletal lead levels in man after end of occupational exposure, Arch. Environ. Hlth.

V. Schütz, A., Skerfving, S., Christoffersson, J.O., Ahlgren, L., and Mattson, S., Lead in vertebral bone biopsies from active and retired lead workers. Submitted for publication.

VI. Schütz, A., Skerfving, S., Christoffersson, J.O., and Tell, I., In press, Chelatable lead vs. lead in human trabecular and compact bone. Sci. Tot. Environ.

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ABSTRACT

The elimination rate for lead from blood was studied in active lead workers, who were removed from exposure due to high blood lead levels (B-Pb), in retired lead workers and ex-lead workers, and in two volonteers exposed during 1 h to a very high air-lead level. The B-Pb decay was well described by the sum of two exponentials, with half-times of about 1 month and 5 y, respectively. However, considerable inter-individual variation in the elimination rates was observed.

Lead in finger bone (finger bone-Pb) was analysed by an X-ray fluorescence method in vivo. The finger-Pb levels rised with time of employment. Levels up to 122 and 135 μg/g were found in active and in retired workers, respectively. B-Pb in retired workers, but not in active ones, was significantly correlated with finger bone-Pb, displaying an endogenous exposure many years after end of occupational exposure. There was a significant correlation between finger bone-Pb and the time-integrated B-Pb.

Finger bone-Pb was monitored in ex-lead workers and in retired lead workers for periods up to 6 y. A decrease in finger bone-Pb was observed in 13 out of 14 subjects. The average half-time was 7 (range 2-oo) y.

In vertebral bone biopsies from active and from retired lead workers, levels up to 155 and 76 μg/g, respectively, were found. Vertebra-Pb increased with time of employment. The ratio between finger bone-Pb and vertebra bone-Pb was significantly higher in the retired workers than in the active ones, suggesting a faster elimination from vertebra than from finger bone. From the difference in the ratios and the length of the post-exposure period, a half-time for lead in vertebra was estimated at 1-2 y, which was also in accordance with the elimination rate calculated from the accumulation pattern.

In mobilization tests with penicillamine, very close correlation was obtained between lead excretion in urine (U-Pb) during 6 h and 24 h, respectively, after ingestion of the chelating agent. Also, the mobilized U-Pb was closely associated with the unmobilized U-Pb. Further, there was a close correlation between U-Pb and B-Pb, as well as with vertebra bone-Pb, while no correlation was obtained as regards finger bone-Pb. Thus, mobilization test is no index of lead in finger bone, and, most probably, neither of any other lead pool in cortical bone.

ABBREVIATIONS

B-Pb	Blood lead level
Bone-Pb	Bone lead level
PCA	Penicillamine
T1/2(1), T1/2(2)	Half-times of the fast and the slow decay phases, respectively, in a two-compartment model on blood lead elimination after end of exposure
U-Pb	Urinary lead excretion
U-Pb(pre-PCA)	U-Pb just before PCA ingestion
U-Pb(6)	U-Pb during 6 h following PCA ingestion
U-Pb(24)	U-Pb during 24 h following PCA ingestion
Y(1), Y(2)	Y-intercepts of the fast and the slow decay curves, respectively, in a two-compartment model on blood lead elimination after end of exposure

INTRODUCTION

Lead is a rather widespread element in the general environment. The average lead concentration in the earth´s crust is estimated at about 15 mg/kg (Handbook of Chemistry and Physics, 1980-81). The most common natural lead mineral is galena (lead sulphide, PbS), which is also the dominant component in the lead ores, from which lead is obtained by a roasting process.

Natural lead, atomic weight 207.2, is a mixture of the four stable isotopes ^{204}Pb (1.5%), ^{206}Pb (23.6%), ^{207}Pb (22.6%), and ^{208}Pb (52.3%).

The isotopes 206, 207, and 208 are the end products of the disintegration series of the natural radioactive elements uranium, actinium, and thorium, respectively. Thus a continuous formation of lead takes place. However, due to the very long half-life of the initial element in each series, the amount formed during thousands of years is entirely negligible in comparison to the amount already present. This formation is, nevertheless, of interest, since gaseous radon constitutes one of the radioactive elements near the terminal end of each of the series. By diffusion, radon may escape from solid materials and from underground waters and thus create a natural air lead pollution in addition to the lead originating from soil dust. At least in industrialized countries, however, this pollution is most probably of minor importance compared to the environmental air pollution.

Sources of lead exposure in Sweden

General environment

Today, in the general human environment, lead exposure occurs through foods (Schütz 1979), including lead originating from lead-soldered cans and lead-glazed pottery, water (Crawford and Crawford 1969; Quinn 1985), alcoholic beverages (Elinder et al 1983; Quinn 1985), tobacco (Quinn 1985), and through ambient air (Schütz et al 1984; Skerfving et al, in press).

Work environment

The production, and the widespread technical use, of lead and lead compounds, implies a considerable lead exposure in many occupational settings, especially where lead or lead compounds are processed at high temperatures or handled in finely divided form, e.g. lead mines, primary and secondary lead smelters, storage-battery factories, brass foundries, potteries, and crystal-glass smelters. Other lead-exposing occupations include handling of lead compounds, e.g. stabilizers and pigments in the polymer industry, painting with red lead, as well as welding and flame cutting of painted objects. In work places, where lead is handled, exposure occurs primarily through inhalation. In addition to the occupational exposure, lead workers may have an increased exposure through consumption of tobacco, snuff, foods, and beverages, which have been contaminated with lead from the work environment.

Absorption

Airways

The pattern of deposition of inhaled lead particles in the respiratory tract is affected by the size distribution of the particles. A large fraction of particles with an aero- dynamic diameter above 5 μm are deposited in the upper airways. Smaller particles, and, during mouth-breathing also an increased fraction of the larger ones, penetrate into the alveolar region of the lungs, where about 20-35 per cent are deposited, while the rest is exhaled (Keho1961; Chamberlain et al 1975 and 1978; Wells et al 1977; Gross 1981; Chamberlain 1985). The rate of absorption of lead from the particles deposited in the lung is dependent upon solubility and thus on the chemical composition of the particles. Particles of low solubility may be phagocytized and introduced into the lymph system.

Gastro-intestinal tract

A fraction of the large lead particles, which have been deposited in the large airways, is cleared by the mucociliary mechanism and swallowed. About one tenth of ingested lead is absorbed from the gastro-intestinal tract (Kehoe 1961; Harrison et al 1969; Chamberlain et al 1978; Moore et al 1980; Rabinowitz et al 1980; Gross 1981; Flanagan et al 1982; Blake et al 1983; Blake and Mann 1983; Heard and Chamberlain 1983; Chamberlain 1985). There are, however, indications of a large inter-individual variation in lead uptake from the human gut (Hursh and Suomela 1968; Blake 1976).

Distribution

Lead is absorbed into the blood plasma (and the lymph, which is later emptied into the blood plasma). A major fraction of lead in plasma is bound to proteins, while the rest is diffusable. Some of the plasma lead represents the ongoing absorption through the lungs and the intestinal tract.

There is a rapid distribution of lead between plasma and extracellular fluid. More slowly, but already within minutes, most of the diffusable lead is incorporated into blood cells. About 99% of the lead content of whole blood is in the red cells, only about 1% in the plasma. The fraction of plasma lead increases somewhat with increasing whole blood levels (Hursh and Suomela 1968; Chamberlain et al 1975; De Silva 1981; Manton and Cook 1984).

The soft tissues, which attain the highest concentrations, are the liver and the kidneys (Piscator and Lind 1972; Barry 1975; Brune et al 1980; Skerfving et al 1983; Gerhardsson et al 1985 and in press). Lead does, to some extent, pass the blood-brain barrier (Barry 1975; Björklund et al 1981; Skerfving et al 1983).

A large proportion of the absorbed lead is incorporated into the skeleton (Gusserow 1861; Grandjean and Holma 1973; Barry 1975; Gross et al 1975). The skeleton contains more than 90% of the body burden of lead (Barry 1975). In lead workers, that fraction may be as high as 99 % (Barry 1975; Skerfving et al 1983).

Considering the turnover of lead, the skeleton may not be a homogenous pool. By analogy with the existence of a very rapidly exchangeable calcium pool, which is readily accessible for the calcium balance (Bauer et al 1961), there is probably also a small but rapidly exchangable skeletal lead pool. In addition, there seems to be at least two other skeletal lead

pools, with considerably slower turnover. One may be contained in trabecular (cancellous, spongy) bone (Barry 1975; Rabinowitz et al 1977; Skerfving et al 1983), which makes up about 20 % of the skeleton, and the other in cortical (compact) bone (Barry 1975; Rabinowitz et al 1977; Ahlgren at al 1980; Lindh et al 1980; Skerfving et al 1983; Somervaille et al 1985).

The relationship between lead exposure and levels in the skeleton is not known. Such knowledge may be important, as it may enable an estimation of past lead absorption, perhaps even make it possible to calculate the absorbed dose, which would be most valuable, both in cross-sectional epidemiological studies on the relationship between long-term exposure and effect/response, and in evaluation of individual patients with symptoms and signs, suspected to be caused by lead exposure.

Excretion

Lead is excreted mainly in the urine. It is excretion primarily through glomerular filtration, as indicated by experimental animal studies (Vander et al 1977). The excretion is rising nonlineary, probably exponentially, with increasing blood lead levels (Tola et al 1973; Manton and Cook 1984, Skerfving et al 1985). This is most probably due to a rising fraction of lead in plasma (see above). When related to whole blood lead levels, urinary lead excretion varies considerably between individuals (Skerfving et al 1985).

Absorbed lead is also excreted by endogenous faecal excretion (Chamberlain et al 1975 and 1978; Rabinowitz et al 1977). At low exposure levels, the fraction eliminated through the faeces is about half of that in the urine, at higher levels probably smaller. Lead is also, to some extent (a few per cent), excreted in sweat (Rabinowitz et al 1977), nails, and hair (Rabinowitz et al 1976).

Toxic effects

Toxic effects of lead have been known since ancient times. The hœmatopoetic and the central and peripheral nervous systems and the kidneys are affected (WHO 1980). Adverse effects in adults are considered to develop at prolonged blood lead levels of about 2.0 µmol/l or higher (WHO 1980). Effects in children probably occur at even lower levels. Lead may also cause fetal damage.

Lead may also affect the mineral metabolism in bone. In children, there are decreasing serum levels of 1,25-dihydroxycholecalciferol (Mahaffey et al 1982) and osteocalcin (Markowitz et al 1986) with rising B-Pb. Further, there are some indications of disturbance by lead of osteoclast function, both in vitro (Rosen 1983) and in vivo (Eisenstein and Kawanoue 1975).

Metabolic model

Theoretically, a compartment model on lead metabolism may contain as many compartments as there are organs, and within the organs, different kinds of tissues, in the body. For practical use, it is important to find a sufficiently simple model, which allows reasonably accurate predictions of the turnover rate of lead in the major lead pools and of the impact of these pools on the most sensitive body functions. The following number of compartments in human lead metabolism have been suggested: One (Fisher 1969), two (Sterling et al 1964;

Black et al 1968; Ahlgren et al 1980; Steenhout 1982), three (Rabinowitz et al 1977; Batschelet et al 1979; Marcus 1979; ICRP 1980), four (Fisher 1969; Chamberlain 1985; Marcus 1985a and b), and even five (Bernard 1977).

There are data on the kinetics of the metabolism of small amounts of lead in man (Rabinowitz et al 1977; Campbell et al 1984; Chamberlain 1985). Also, some information on the pattern of elimination of lead from blood after end of occupational (McRobert 1973; Ahlgren et al 1980; O'Flaherty et al 1982 and 1986; Lynam and Nelson 1982; Kang et al 1983) and other exposure (Kehoe 1961; Schlegel and Kufner 1979; Stuik 1974; Griffin et al 1975; Gross 1975; De Silva 1981) has been reported. However, the amount of information was often limited and the models used for the analysis sometimes fairly crude. Thus, more information is needed, especially as blood-lead levels are the main tool used for biological monitoring of lead exposure.

The lead, which has been deposited in the skeleton, is released into the blood stream by the continuous turnover of the bone tissues. This release causes an elevation of the blood-lead level during many years after end of excessive exposure (Prerovska and Teisinger 1970; Grandjean and Kon 1981; Hesley and Wimbish 1981; Mindus and Kolmodin-Hedman 1981; Neri et al 1983). Evaluation of recent lead exposure from the present blood-lead level may thus be erroneous. However, the impact of the skeletal lead pool on the blood lead level has not been adequately known.

The risk of poisoning, at a particular exposure level, may vary between individuals, due to inter-individual differences in metabolism. There are indications of such variations in the metabolism of methylmercury (Skerfving 1974), cadmium (Welinder et al 1977), and chromium (Welinder et al 1983) in man, and differences may be important also for lead (Marcus 1985a). In dogs, there are indications of a large inter-individual variation in the kinetics of lead metbolism in the skeleton (Fisher 1969). However, the data on lead are not conclusive. Thus, it is of great importance to bring forward more detailed knowledge on inter-individual differences in lead metabolism in man to make possible a more accurate estimate of risks in groups and on an individual basis.

Chelatable lead

Lead in the body is chelatable by etylene diamine tetraacetic acid (EDTA; e.g. Chisholm 1976) and d-penicillamine (PCA; Ohlsson 1963). EDTA is also a strong chelating agent for calcium, and is administered as the calcium disodium complex to avoid excessiv excretion of calcium.

Intake of these substances results in increased urinary lead excretion ("mobilization test"), which may be used for diagnosis of an increased body burden of lead and risk of poisoning. It has also been claimed that the chelatable lead reflects the skeletal lead pool (Wielopolski et al 1986). It is, however, far from clear that this is the case in man.

AIMS OF THE STUDY

1. What is the pattern and kinetics of blood lead after end of exposure?
2. Are there inter-individual variations in blood-lead kinetics?
3. What are the lead levels in the skeleton of lead workers?
4. How does skeletal lead affect blood lead?
5. Are there kinetically different skeletal lead pools?
6. What is the turnover rate of lead in the skeleton?
7. Are there inter-individual varations in kinetics of the skeletal lead?
8. Is the skeletal lead an index of the "accumulated" lead dose?
9. Does a lead mobilization test indicate the skeletal lead level?

MATERIALS AND METHODS

Subjects studied

The results of this thesis are based on repeated observations of totally 131 males with a history of occupational lead exposure, and on two volonteers, who suffered a single, short, heavy exposure.

Of the lead workers, 75 were active, 33 were retired, and 23 had left lead work for other reasons. Ninety-five were, or had been, employed at a secondary smelter for lead, tin, and copper alloys, 20 in a storage-battery plant, eight in a brass foundary, four in demolition work (scrap metal), two in spray-painting, one in paint production, and one in wire lead coating. The average exposure time was, for the whole group of lead workers, 16 y, (range 0.1- 46 y).

A "back-ground" blood-lead level was established in 47 males with no occupational lead exposure, who were working outside the lead-polluted industrial area (**II**).

A reference material on lead in bone biopsies was sampled from the two volonteers and from 12 autopsy cases of sudden death with no history of occupational lead exposure (**V**).

Blood lead levels

Two 10 ml samples of venous blood was obtained from the cubital vein in metal-free heparinized evacuated tubes. Five ml samples were wet-ashed and lead was complexed with dithizone, extracted, and determined by flame atomic absorption spectrophotometry (AAS) (**I**; Schütz 1979; Schütz et al 1984). Each sample was analysed twice. The detection limit was 0.05 µmol/l (10 µg/l).

Each analytical series contained six samples, and two blanks containing reagents only, and four "normal" blood samples, two of them with standard lead addition. Each sample was analysed in duplicate, in different series.

The coefficient of variation, as calculated from duplicate analyses of 57 samples containing 0.5 µmol/l or less was 6.6%, in 25 samples containing 0.5-1 µmol/l 3.9%, in 58 samples containing 1-2 µmol/l 2.5%, and in 60 samples containing 2-3.5 µmol/l 2.0%.

The accuracy was tested twice each year in an inter-Nordic laboratory calibration program, with 6-19 (mean 12) laboratories participating at each occasion. The regression function of our results (Y, µmol/l) on the average result (X) of the other approved laboratories was $Y=1.008X-0.052$. Our results in the 115 samples (range 0.2-5.6 µmol/l) averaged 96.3% (range 80-112%, 62% within 95-105%) of the mean of the other laboratories. Further, we participated in the U.K. External Quality Assessment Scheme since 1983. In the 38 samples analyzed in the period when most of the active lead workers were studied, our results, by single analysis of 3-4 g samples, averaged 99% (range 90-120%, 74% within 95-105%) of the mean (2.4 µmol/l) of about 90 participating laboratories. Neither quality control series displayed any time trend.

The method described above has been used during the last 12 years in the study of blood lead kinetics. In the ex-lead workers studied for longest time (**II**), blood lead was initially determined by a colorimetric method, after extraction with dithizone in chloroform. The ashing procedure and the set-up of blanks, standards and samples was the same as in the

flame-AAS method. A separation step involving co-precipitation of lead with calcium phosphate from an oxalate-containing solution of about pH 5 was used to eliminate colorimetric disturbances from iron in the chloroform-dithizone extract. Potassium cyanide was added to the ammonia-citric acid buffer solution to avoid interferences from e. g. cadmium, copper, and zinc. The detection limit of the method was 0.05 µmol/l. During a transition period, when the method was changed, samples were analysed by the colorimetric, as well as by the flame-AAS method. The values obtained by the colorimetric method were somewhat higher (mean105%, SD=5.8%, n=24) in the concentration range 0.3-1.9 µmol/l, while there was no difference (mean 100%, SD=4.8%, n=34) between the methods at higher concentrations (figure 1).

In a group of lead workers, whose entire period of occupational lead exposure was covered by blood lead monitoring (III), the blood samples were analysed at another laboratory during a period of the study. Thus, in addition to the flame-AAS method described above, two other methods were employed during different periods. The laboratory was participating, and was approved, in the inter-Nordic laboratory cali- bration program mentioned above.

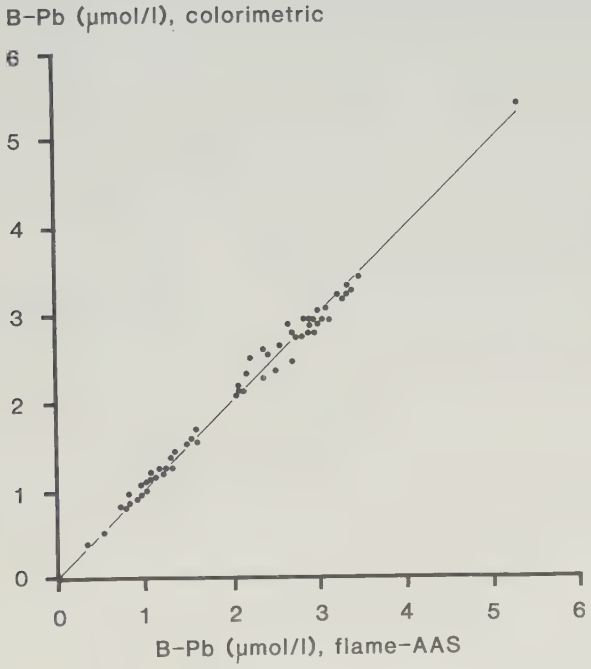


Fig. 1. Comparison between colorimetric and flame-AAS method for determination of lead in blood.

Time-integrated blood lead levels

In active lead workers, whose blood lead levels had been monitored during the entire period of occupational lead exposure, usually four times yearly, a time-integrated blood lead level, (µmol/l x year), was estimated graphically.

Skeletal lead levels

Finger-bone

Lead level in bone (bone-Pb) was analysed in *finger-bone* by an X-ray fluorescence (XRF) method *in vivo* . The middle phalanx of the left hand forefinger was irradiated by gamma rays from ^{57}Co and the secondary characteristic K alpha X-rays from lead were measured by an energy dispersive germanium detector (**III**; **IV**; Ahlgren et al 1976 and 1980). The total absorbed energy and the effective dose equivalent were at the order of 1×10^{-2} mJ and 0.1 µSv, respectively.

The detection limit was 20 µg/g (signal level corresponding to three times the square root of the number of background counts). The precision of the method was about 15%. The accuracy was good, when a phalanx from a dead lead worker was analysed *in vitro* by the present method, and the results were compared with the results of chemical analysis (**III**).

Vertebral bone

Samples of vertebral bone were obtained by needle biopsy from the spinous process of one of the lumbar vertebrae L4 -L6 (Radner 1962; V). Fresh and dry weight was determined. After a combined wet and dry ashing procedure, *lead* was analysed by electrothermal AAS, using the technique of standard addition (**V**).

A minimum of three reagent blanks was included in each analytical series of biopsy samples. As an average, one reagent blank and one lead standard was included on each third biopsy sample and treated parallell to the biopsies throughout the ashing procedure. Addition of calcium (1 mg) to the blanks or the water standard samples, prior to ashing, prevented loss of lead through volatilization during the dry ashing step. The lead content in the amount of calcium added was analysed and was found to be negligible.

To compensate for differences in bone-Pb between different biopsy samples, due to variation in the mass fraction of proper bone tissue, the lead content was related to the *calcium* content of the samples.

The detection limit for lead was about 10 ng per sample, corresponding to a bone-Pb of about 1 µg/g fresh weight or about 10 µg/g calcium.

The precision of sampling and analysis was estimated on two sets of "biopsy" samples taken at autopsy from two lead workers, A and B, respectively. The coefficient of variation was in subject A 14% (n=8; mean 73 µg/g; V) and in subject B 21% (n=3; mean 52 µg/g), as calculated on fresh weight. When the ratios lead/calcium were considered, the coefficient of variation improved to 5% and 9%, respectively (table 1).

The accuracy of the analytical step was investigated by checking the recovery of lead

standard added, prior to the ashing procedure, to a number of "biopsy" samples taken at autopsy from two subjects without known lead exposure. Thus 0.50 µg of lead was added to six out of 12 samples and 1.00 µg to three out of seven samples. The average recovery was 0.53 µg and 0.99 µg, respectively (table 1). The recovery was calculated as the difference between samples with standard addition and the average of the samples without addition. The amounts of lead standard added corresponded to a bone-Pb of about 30 µg/g and 60 µg/g, respectively, when the average fresh weight of the biopsy samples was considered.

The accuracy was further checked by analysis of "macro" samples from the same spinal processes of subjects A and B, from which the "biopsy" samples were taken for the estimation of the precision. Thus, after a similar ashing procedure, as was used for the biopsy samples, the ash residue was disolved in concentrated nitric acid and diluted to 25 ml. Suitable fractions of these samples were then analysed by the method used for wet-digested blood samples. The average lead content per gram calcium in the biopsy samples from subject A was 750 µg as compared to 800 µg in the "macro" samples (two spinal processes from subject A analysed; each divided in two pieces). The corresponding values in the samples from subject B was 590 µg and 530 µg (one piece of spinal process analysed) (table 1). The total fresh weight of the "macro" samples analysed was 7.40 g from subject A and 1.61 g from subject B.

In addition, two commercial standard reference materials, *H-5 animal bone* from International Atomic Energy Agency (IAEA), Vienna, and *1571 orchard leaves* from National Bureau of Standards (NBS), Washington D.C., were analysed. Five 30 mg samples from each of the pulverized reference materials were analysed by the method used for the biopsy samples. Prior to ashing, 1 mg of calcium were added to the orchard leave samples.

The average lead level found in the *animal bone* was 3.09 µg/g, as compared to the certified value of 3.1 µg/g. The average level found in the *orchard leaves* was 45.6 µg/g, while the certified value was 45 µg/g (table 1). Agreement with the certified values was obtained,though the size of the analysed samples was far below the minimum sample size, 100 and 250 mg, respectively, which is recommended to avoid variation in the results due to inhomogenity in the standard materials.

The results of the precision and the accuracy tests summarized in table 1, thus demonstrates a satisfying accuracy, as well as precision, at the lead level present in biopsies.

The fresh weight of the biopsy samples was 7.7-23.6 mg. The calcium concentration was 3.4-19.4 (mean 9.9)%, calculated on fresh weight, thus indicating a considerable variation in the ratio between bone tissue and bone marrow. The influence of this variation was minimized by using the ratio µg Pb/g Ca.

Calcium was analysed by a routine flame AAS technique (wavelenght 422.7 nm) after dilution of the samples with 2% nitric acid containing 1% w/v of lanthanum to avoid suppression of signal sensitivity, due to the presence of i.a. phosphate.

The precision and accuracy of calcium analysis by the present method is shown by the results on the five samples of H-5 animal bone. An average calcium concentration of 22.4% (SD=1.3; range 21.2-24.2) was obtained. The certified value was 21.2% (95% confidence interval: 20.4- 22.0).

Table 1. Testing accuracy and precision of the method for lead analysis in biopsies.

Test sample	Certified value	Result of analysis Mean	SD	Range	N
IAEA H-5 animal bone	3.1 µg/g (2.6-3.7)[1]	3.09 µg/g	0.23	2.84-3.44	5
NBS 1571 orchard leaves	45 µg/g (42-48)[1]	45.6 -"-	6.1	39.0-52.0	5
"Normal" biopsy +Pb	0.50 µg[2]	0.53 µg[3]	0.03	0.49-0.57	6
"Normal" biopsy +Pb	1.00 µg[2]	0.99 "[3]	0.09	0.93-1.09	3
Spinal process, subj. A					
-"macro" sample	-	800 µg/g Ca	60	720-860	4
- biopsy	-	750 - " -	40	660-780	8
Spinal process, subj. B					
-"macro" sample	-	530 - " -	-	-	1
- biopsy	-	590 - " -	40	570-640	3

1) 95% confidence interval.
2) Lead added to biopsies from occupationally unexposed subject.
3) Recovery of added amount. Initial level determined and subtracted.

Lead mobilization test

The lead mobilization test was performed according to Ohlsson (1963), with some modifications. After at least two hours fasting, the subject collected a urine spot sample and ingested 0.5 g of d-penicillamine (Cuprimine®). All urine was collected for 6 h in another bottle, and then, in a separate bottle, for an additional 18 h, to obtain the excretion during 24 h. The subjects were instructed to empty, as completely as possible, the total volume of urine accumulated in the bladder, before the mobilization test and at the end of each sampling period.

In the active workers, the mobilization test was usually started within less than 15 h after a work shift, and the 24 h sampling usually included the next work shift.

Urine was collected in acid-washed polyethylene bottles. The volume, density, and creatinine contents were determined.

Urinary lead level was analysed in duplicate 5-20 ml samples (depending upon the expected concentration) by the method also used for blood lead analysis. The detection limit was 0.01 µmol/l. The precision, as calculated from duplicate analyses, was 5.3% in the range 0.05-0.5 µmol/l (N=48), 3.5% in the range 0.5-1.0 µmol/l (N=51), 3.3% in the range 1.0-2.0 µmol/l (N=17), and 2.7% above 2.0 µmol/l (N=11).

Creatinine levels in urine were determined according to a modified kinetic Jaffé method (Lustgarten and Wenk 1972). The method error at 10 consecutive analyses of one sample was 2%.

Mathematical calculations

The decay of B-Pb after end of exposure in study II was described by fitting one, the sum of two, or the sum of three exponential functions to the data on observed B-Pb in individual subjects, by use of a Gauss-Newton iterative nonlinear least square method. A back-ground B-Pb of 0.3 µmol/l (the value obtained in the 47 reference subjects) was used in all models. The fit between the observed B-Pb values and the calculated decay curve was expressed as the explained fraction of the total variance (R^2,%).

In study I, the curve-fitting involved a weighted least square method, which gave a somewhat different fit as compared to that employed in study II. The T1/2 values presented in study I have thus been recalculated according to the method used in study II in order to make them comparable to the results in study II. The elimination rate of lead from finger-bone (IV) was calculated by a nonweighted least square method.

To describe the accumulation of lead in the slow compartments as reflected in blood (**II**) and in bone (**V**), a function of the type $Y(t)=A(1-e^{B \times t})$ was fitted to the data by use of the last square method (A is a scale constant, B an elimination constant, and t is the time of exposure).

Statistics

Almost only non-parametric statistics (Mann-Whitney U-test, Wilcoxon´s matched-pairs signed-ranks test, Spearman´s rank correlation test, r_S) were employed. In some cases, single or multiple linear regression analysis was performed. All p-values are two-tailed. "Statistically significant" refers to $p \leq 0.05$.

RESULTS

Decay of blood lead level after end of exposure

Subjects without occupational exposure

Two volonteers, A and B, after having inhaled during 60 minutes about 100 mg of an aerosol of lead oxides and sulphate, were followed for 215 d and 500 d, respectively (**I**). Within these periods, B-Pb decreased to the pre-exposure values. The T1/2 values reported in paper I have been recalculated by use of the same mathematical model as was later used in paper II.

In both subjects, two phases in the B-Pb decay were clearly distinguished. The T1/2 of the both phases and their 95 % confidence limits were, in subject A 1.4 (1.0-2.6) and 50 (43-59) d and in subject B 0.9 (0.5-7.3) and 56 (48-67) d respectively. The fit between the decay curves and the observed B-Pbs, as expressed by the R^2-value, was in both subjects 99%.

The B-Pb decay was also described by another two-compartment model, with T1/2 of the slow compartment fixed at 5 y, as calculated from the data on ex-lead workers (**II**). Then the T1/2 of the fast compartment was 27 (17-62) d in subject A and 44 (32-71) d in subject B, and the R^2-values were 94% and 97%, respectively.

Lead workers

Long term B-Pb decay was studied in 23 *ex-lead workers* up to 15 y (median 11; range 1.7-15) after end of exposure (**II**).

Although two phases could be distinquished in most decay curves, only in three of these subjects, there was a sufficient number of observations soon after end of exposure (at least four during the first two months) to make an estimation of the decay rate of the fast compartment of a two-compartment model meaningfull.

Short term B-Pb decay was studied by frequent blood sampling in 17 *active lead workers* during their removal from lead exposure due to high B-Pbs (**II**). Totally 19 occasions of removal were studied; two subjects were removed twice. The median observation time (=time of removal) was 155 (range 48-239) d. Also in this group, there was a clear evidence of two phases in the decay curves, but the observation period was far to short to allow determination of the decay rate of the slow compartment.

In two subjects, temporarily removed from exposure, blood samples were taken more frequently during the first days after end of exposure in order to reveal any initial very fast decay phase, as that seen in the two occupationally unexposed volonteers. However, in these temporarily removed subjects, no such phase was discovered (figure 2).

The following data on B-Pb kinetics in lead workers were obtained by combining the observations on B-Pb decay in the both groups of lead workers (**II**):

The observed individual B-Pb data fitted significantly better to a two-compartment model than to a one-compartment one. The fit was only marginally better when a three-compartment model was used. Also, in many of the subjects studied, the small number of B-Pb

observations resulted in such wide 95% confidence intervals of the half-times, that in six subjects infinity was included already within the interval of the second half-time. The main calculations on the B-Pb kinetics were thus made on a two-compartment model.

The T1/2(1) was calculated at a median of 29 (range 7-63) d (using 5 y as an estimate of T1/2(2), see below) in the temporarily removed subjects. The half-time of the slow compartment was calculated at a median of 5.6 (range 2.3-27) y (using 30 d as an estimate of T1/2(1) in all but those three cases, where the number of observations during the first months of the exposure-free period were insufficient). Significant inter-individual differences appeared in the B-Pb decay rates.

The influence of varying T1/2(2)s on the T1/2(1) was studied by recalculating T1/2(1) in the temporarily removed workers, using different T1/2(2) values. If T1/2(2) was assumed to be 2.3 y and 16 y, respectively, median T1/2(1)s of 23 (range 3-64) d and 29 (range 8-82) d, respectively, were obtained. Thus, a considerable variation in the T1/2(2) estimate had only a limited influence on the calculated T1/2(1) values.

At the end of exposure, a major fraction of B-Pb was associated with the slow compartment, as characterised by the Y-intercept of the slow decay phase, Y(2). In the temporarily removed workers, it amounted to 57%, and in the ex-lead workers to 68% of the total B-Pb.

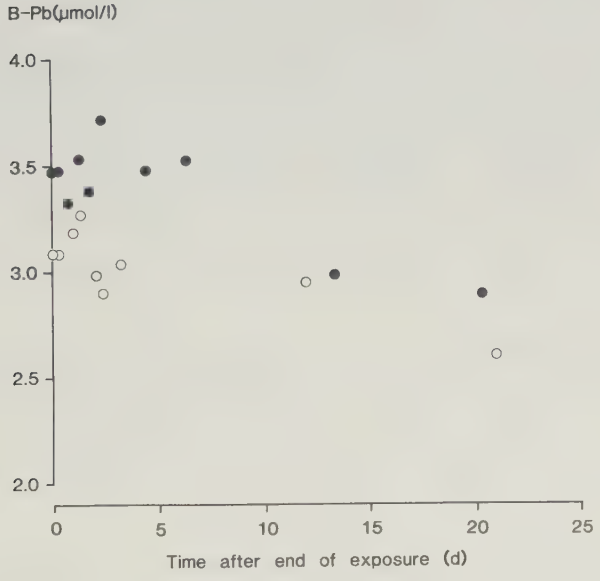


Fig. 2. Frequent observations of blood lead level in two subjects temporarily removed from lead exposure.

In the active lead workers, the median B-Pb was 3.5 (range 2.8-6.7) μmol/l at the time of removal from exposure. In 16 of the 19 removals studied, B-Pb decreased at or below 2.5 μmol/l during the observation period. The median time for the decrease was 48 (range 14-190) d and the median Y(2) in these cases was 1.8 (range 0.9-2.4) μmol/l. The three remaining cases did not reach 2.5 μmol/l during observation periods of 118, 189, and 209 d, respectively. Their Y(2)s were as high as 2.3, 2.6, and 2.7 μmol/l, respectively.

Only in nine cases did B-Pb decrease to or below 2.0 μmol/l during a median observation time of 97 (range 70-160) d. The median Y(2) in these cases was 1.6 (range 0.9-1.8) μmol/l. In those, who returned to lead exposed jobs before reaching the 2.0 μmol/l level, the corresponding value was 2.2 (range 1.7-2.7) μmol/l. (Note, that the Y(2)-values do not include the back-ground B-Pb of 0.3 μmol/l.)

Studies of the skeletal lead pool

Finger bone

The finger bone lead level (bone-Pb) was studied cross-sectionally in 75 active and in 32 former (mostly retired) lead workers (**III**). In another, longitudinal study on 14 former lead workers, the finger bone-Pb was determined repeatedly during periods up to six years (**IV**). In eight of the subjects in the latter study, the first measurement was made within the first exposure-free year. In the remaining six subjects, the measurements were performed from year 7 to 13 after end of exposure. Data on age, time of exposure, exposure-free period, bone-Pb, and B-Pb at the first measurement is summarized in table 2.

Table 2. Summary of data on subjects with finger bone lead measurements. Mean or median (*) values and ranges are indicated.

Subjects (Study No.)	N	Age (y)	Exposure-time (y)	Exposure-free (y)	Bone-Pb (μg/g)	B-Pb (μmol/l)
Active (III)	75	46 (19-65)	10 (0.1-37)	0	43* (<20-122)	2.6 (0.8-5.0)
Retired (III)	32	68 (53-80)	24 (4 -13)	4.3 (1-13)	59* (<20-135)	1.2 (0.4-1.8)
Ex-lead (IV)	8	61 (49-64)	24 (10-38)	0.1* (0-1.0)	93[1)] (47-141)	2.0 (1.3-2.8)
Ex-lead (IV)	6	56 (37-72)	26 (3 -45)	7 (7-7)	72[1)] (27-95)	1.1 (0.6-1.4)

1) The values refer to end of exposure, as derived from the individual decay functions, Bone-Pb(t) = A x $e^{k \times t}$, where k is the decay rate constant; t is time.

There were significant correlations between finger bone-Pb on the one hand and the time of exposure (r_s=0.49, p=0.002), and the time integrated B-Pb (r_s=0.41, p=0.003) on the other (**III**). There was no significant effect of the length of the exposure-free period on bone-Pb in the retired workers.

In the retired workers, but not in the active ones, there was a significant linear correlation between B-Pb and bone-Pb (r_s=0.42, p=0.01). In 33% of the retired workers, B-Pb was above 1.5 µmol/l (**III**).

In the longitudinal study (**IV**), all but one of the 14 ex-lead workers, displayed a decrease in bone-Pb. The half-time, as calculated from the average decay rate constant for all subjects, was about 7 y. The individual half-times ranged 2 - ∞ y. The fit between the individual decay curves and the measured bone-Pb values was, however, far from good, as judged from the coefficients of linear correlation between log(bone-Pb) and time (median r= -0.43; range -0.85 to +0.06). The method error in the bone-Pb measurement was probably the major reason for the limited correlation.

The slow compart of a two-compartment model of the decay of B-Pb in the same subjects had an average half-time (T1/2(2)) of about 7 y, as calculated from the average decay rate constant. The individual half-times ranged 3.5-18 y. There was, however, no association between the individual´s half-times in blood and in bone.

The slow compartment of B-Pb decay, as reflected by Y(2), was significantly correlated with finger bone-Pb (r_s=0.36, p=0.01). At a particular finger bone-Pb, the temporarily removed workers had on average 1.0 µmol/l higher Y(2) than had the retired ones. Multiple regression analysis displayed an impact on Y(2) by 0.008 µmol/l per µg/g of bone-Pb.

Vertebral bone

Biopsies were obtained from 27 active lead workers, from nine retired workers (on an average 2.6 (range 2.3-5.6) y after end of lead exposure), and from 14 occupationa- lly unexposed reference subjects(**V**). Also, finger bone-Pb and B-Pb was determined in all subjects except in 12 of the references. In 16 of the active workers, a time integrated B-Pb was calculated for the whole exposure period. Further, in seven of the active and in one of the retired workers, the decay of the blood lead level was studied during a period of temporal removal and after retirement, respectively (**II**).

The data on the subjects studied are summarized in table 3.

Table 3. Summary of data on vertebra bone lead measurements. Group A are active lead workers, group B retired lead workers, and group C reference subjects. Averages or medians (*) and ranges are indicated.

	N	Age (y)	Exposure-time (y)	Bone-Pb Vertebra µg/g	Vertebra µg/g Ca	Finger µg/g	B-Pb (µmol/l)
A	27	46 (26-65)	11 (0.6-39)	29* (2-155)	370* (30-1120)	40* (<20-115)	2.3* (0.8-7.2)
B	9	67 (61-71)	29 (6-46)	19* (5-76)	250* (60-700)	83* (<20-135)	1.4* (0.7-1.7)
C	14	59 (34-88)	0	1.3* (1-4)	13* (8-40)	<20[1)] (<20-<20)	0.3[1)] (0.3-0.3)

1) Only two subjects measured.

There was a significant correlation between vertebra bone-Pb and the time of employment (r_S=0.77, p=0.001), as well as with the time-integrated B-Pb (r_S=0.93, p=0.001). The subject with the fastest accumulation rate of lead in vertebra accumulated almost 50 μg/g per year. The accumulation rate of lead in finger bone was, in the same subject, about 15 μg/g per year.

A very crude estimate of the elimination rate of lead from vertebral bone was obtained by fitting an accumulation curve to the data of the active workers. A half-time of about 2 years was thus estimated. However, due to the probably considerable differences in exposure intensity between the workers, the error in this estimate may be considerable. Further, it does not take into account the continuously increasing endogenous exposure from the skeleton, which results in an increase in B-Pb, and thus an increased re-deposition of lead in vertebra.

In individuals, there was generally a difference between vertebra bone-Pb and finger bone-Pb. The ratio between finger bone-Pb and vertebra bone-Pb was significantly higher in the retired workers than in the active ones (median ratio 4.0 vs. 1.3; p=0.01). There was, however, no association between these ratios and time of exposure in the active workers, nor with the time after end of exposure in the retired ones.

A crude estimate of about 1.5 y for the half-time of lead in vertebral bone can be calculated by using the data on the median ratios in active and retired workers, respectively, an exposure free period of 2.6 y in the latter group, and assuming a half-time of 7 y for lead in the finger-bone (**IV**).

B-Pb correlated very closely with vertebra bone-Pb in the retired workers (r_S=0.93, p= 0.001), but there was also a significant correlation in the active ones (r_S=0.44, p=0.01). At a particular vertebra bone-Pb, the B-Pb was higher (about 1.5-2 times) in the active workers as compared to the retired ones.

The ratio between B-Pb and vertebra bone-Pb was significantly higher in the active than in the retired workers. In the latter group, there was also a correlation between B-Pb and finger bone-Pb (r_S=0.85,p=0.002), while no such correlation was present in the active workers (r_S=0.28. p=0.08).

There was a close correlation (r_S= 0.96, p=0.0.001, n=8) between the Y-intercept of the fast phase, Y(1), of the blood lead decay curves (**II**), and vertebra bone-Pb in the lead workers (figure 3). The Y-intercept of the slow decay phase, Y(2), exhibited no significant such correlation (r_S=0.13, p=0.4). As regards finger bone-Pb, there was no correlation with Y(1) or Y(2). The median exposure time for these subjects was 15 (range 3-38) y.

Lead mobilization test

A single dose of penicillamine (PCA) was given perorally to 21 active and 14 retired lead workers and to two occupationally unexposed volonteers (**VI**). The median post-exposure time for the retired workers was 2.9 (range 1.0-5.6) y.

In 11 of the active and in 10 of the retired lead workers and in the two volonteers, vertebra bone-Pb and finger bone-Pb were determined.

In the samples collected during the first 6 h of the mobilization test, the median lead concentration was 3-4 times higher, both in the active and in the retired workers, as compared to the pre-mobilization samples. The excreted amount during the first 6 h, U-Pb(6), accounted for more than half of the total amount of lead excreted during 24 h, U-Pb(24). There was a very close correlation (r_s=0.98, p<0.02) between U-Pb(6) and U-Pb(24).

A comparison between U-Pb(6) and the excretion prior to the mobilization (U-Pb(pre-PCA)) was made. There was a close correlation between these values in the active workers (r_s=0.88, p<0.002), and, still better in the retired ones (rs=0.97, p<0.002, figure 4). The association seems to be rectilinear.

The B-Pb was significantly (p=0.03) higher in the active workers (median 2.2, range 0.8-4.0 µmol/l) than in the retired ones (median 1.5, range 0.7-1.9 µmol/l).

There was a close correlation between U-Pb(pre-PCA) and B-Pb. The correlation coefficient (r_s) was 0.91 (p<0.002) in the active and 0.92 (p<0.002) in the retired workers (figure 5). In both groups, the linear correlation coefficient improved when the logarithmic values of U-Pb(pre-PCA) were used; in the active workers from 0.74 (p=0.0001) to 0.88 (p<0.00002), and in the retired ones from 0.89 (p=0.0002) to 0.94 (p=0.00002).

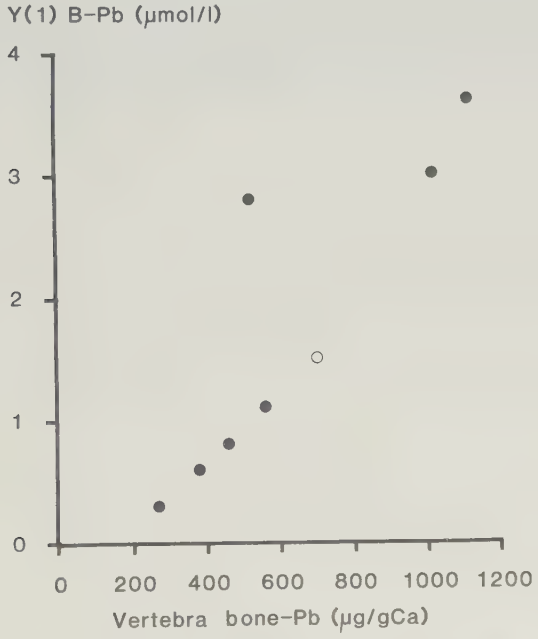
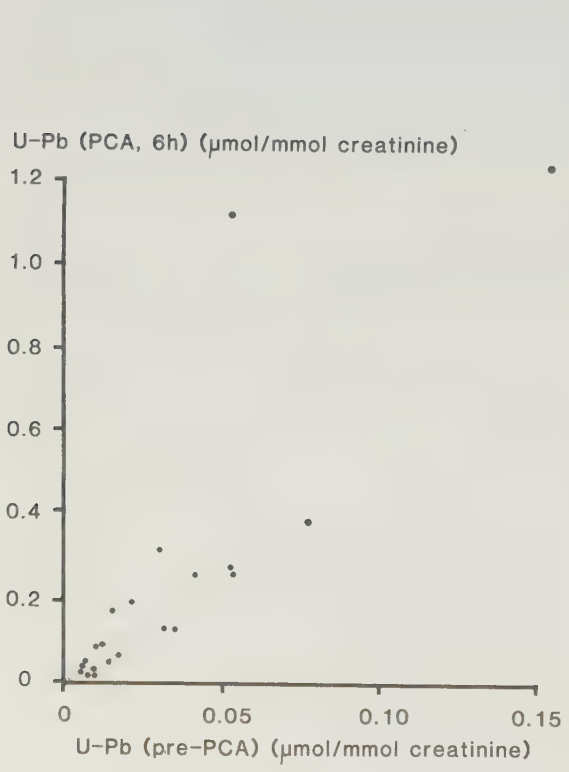


Fig. 3. Relationship between lead level in vertebra (vertebra bone-Pb) and the fast compartment (Y(1)) in a two-compartment model fitted to the decline of blood-lead levels (B-Pb) after end of exposure. Closed symbols: active lead workers; open symbol; retired lead worker.

U-Pb(6) was closely correlated with B-Pb, both in active (r_S=0.93, p<0.02) and in retired (r_S=0.85, p<0.02) workers. The association between U-Pb(6) and B-Pb was obviously exponential (**VI**, figure 2). The linear correlation coefficient, as calculated on the total population, thus improved from 0.45 to 0.84 when ln(U-Pb(6)) was used instead of U-Pb(6). At a particular B-Pb, the U-Pb(6) was the same in the active as in the retired workers.

There was a close correlation between U-Pb(6) and vertebra bone-Pb, in active (r_S=0.91, p<0.02), as well as in retired (r_S=0.97, p<0.02) workers. At a particular vertebra bone-Pb, U-Pb(6) was about three times higher in the active workers (p<0.002).

Considering finger bone-Pb, there was no correlation with mobilized lead in urine in neither active (r_S=-0.16, p>0.05) nor retired (r_S=0.43, p>0.05) workers, nor was there any correlation in the total population studied (r_S=-0.21, p>0.05).



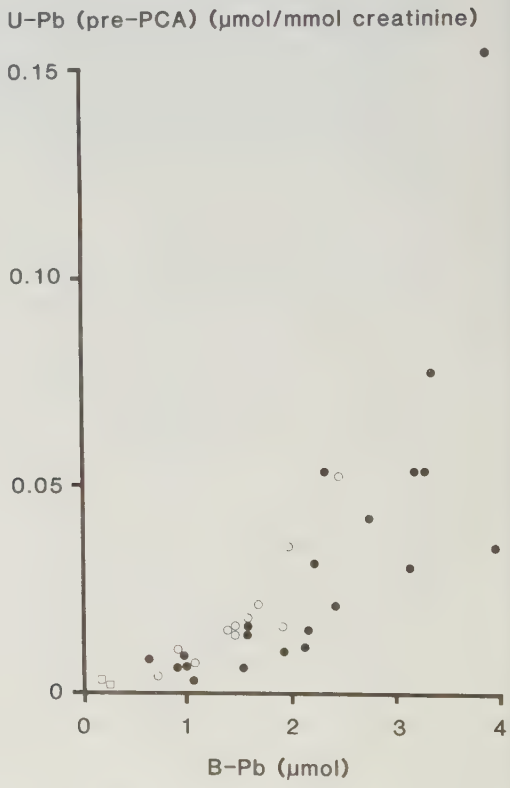


Fig. 4. Urinary lead excretion prior to penicillamine ingestion (U-Pb(pre-PCA)), and during 6 h after PCA ingestion. Closed symbols: active lead workers; open symbols: retired lead workers.

Fig. 5. Lead levels in blood (B-Pb) and urinary lead excretion prior to penicillamine ingestion (U-Pb(pre-PCA)). Closed symbols: active lead workers; open symbols: retired lead workers.

DISCUSSION

Kinetics of turnover of lead in the blood

Sometimes power functions have been employed for description of the turnover of lead in the body. However attractive mathematically, as regards possibilities to obtain a nice fitting to empirical data, power functions have a limited value from a conceptual point of view. In that sense, linear exponential models are much more useful.

Linearity

Is a linear model applicable to blood lead kinetics? There are data indicating nonlinearity, e.g. as regards the relationship between lead levels in air (Hammond et al 1981; Chamberlain 1983 and 1985; Brunekreef 1984; Colombo 1985), diet, or water (Thomas et al 1979; Nutrition Foundation Expert Advisory Committee 1982; Department of the Environment 1982; Bruaux et al 1985) on the one hand, and B-Pb on the other. Nonlinearity is also indicated for the relationship between lead levels in plasma and blood cells, as well as between blood and urine (**I**; Tola et al 1973; Manton and Cook 1984; Skerfving et al 1985).

On the other hand, data on blood lead does not indicate concentration-dependent kinetics, as similar elimination rates have been noted both at very low (Rabinowitz et al 1977; Chamberlain et al 1978; Campbell et al 1984) and at very high (**II**; Chamberlain and Massey 1972; McRoberts 1973) levels.

Number of compartments

What number of compartments should be employed on blood lead decay curves? There is some data indicating that there are many compartments in the body, and that e.g. blood may not be fully representative for the concentration in the soft tissues. Thus, in animal experiments (Hietanen et al 1980; Aungst et al 1981), there is no constant relationship between levels in blood and in soft tissues. Also, the accumulation in liver and kidney is higher than in blood, while it is lower in the central nervous system (CNS). The peripheral nervous system may accumulate considerably more lead than the CNS.

Limited data on deceased humans, without and with occupational lead exposure, also indicates that there is no constant relationships between levels in blood, liver, kidney, and CNS (Grandjean och Holma 1973; Barry 1975; Brune et al 1980; Skerfving et al 1983; Gerhardsson et al 1985 and in press) and that these organs may thus not belong to the same compartment. Further, there are data (Barry 1975; Rabinowitz et al 1977; Skerfving et al 1983), including data from the present study (**V**), which indicates that there is more than one lead compartment in the skeleton.

As mentioned above (**Introduction**), different authors have used models with varying numbers of compartments. In a most detailed study of lead metabolism in man, Rabinowitz et al (1977) analysed data from experiments in humans exposed to relatively low dietary doses of labelled lead. They found a better fit of a three-compartment linear model, as compared to a two-compartment one. However, the turnover rates and the sizes of two of their compartments were very similar.

In this study (**II**), one, two and three compartments were applied on sets of curves for

B-Pb decrease after exposure in lead workers. There was a good fit for a two-compartment linear model, while three compartments gave an only marginally better fit. It thus seems that, at least from a practical point of view, and within the concentration and time intervals studied, a two- compartment linear model is useful, especially for evaluation of B-Pb data in a concentration range relevant for lead workers.

Decay rates

What is the B-Pb decay rate? The kinetics in a linear exponential model may be expressed as transfer constants, mean residence times, or half-times. In this study half-times was chosen, as it is conceptually simple.

In lead workers, the elimination rate of the fast compartment in the present study was about one month (**II**). This is in accordance with the half-time of 3-4 weeks in the soft tissue compartment(s), found in radiotracer studies in man (Rabinowitz et al 1977; Chamberlain et al 1978; Campbell et al 1984).

Another, much faster initial B-Pb decay phase, was present in both of the two occupationally unexposed subjects, who were studied after a short, very heavy exposure (**I**). No such phase was seen in two lead workers studied frequently after removal from exposure. This very fast initial decay reflected, most probably, a distribution of lead from blood into the soft tissue pool, which was practically empty in these subjects. In the lead workers, an equilibrium was already established.

In subjects without occupational exposure, the whole blood lead levels may be remarkably stable over time (Delves et al 1984). However, after a heavy exposure, the level may rise by ten-fold within a few hours (**I**; De Silva 1981). After a less dramatic rise of the exposure intensity, the lead level in blood increases gradually, usually to reach a steady state after weeks to months (**III**; Kehoe 1961; Tola et al 1973; Stuik 1974; Griffin et al 1975; Benson et al 1976; Forni et al 1976; Gross 1981; Lerner et al 1982; Neri et al 1983). This is generally compatible with a half-time of about a month.

A half-time of about a month is also compatible with (usually far less extensive) information on the elimination of lead from blood after end of exposure, if a two-compartment concept is applied to the data reported in various studies (Kehoe 1961; Chamberlain and Massey 1972; McRoberts 1973; Stuik 1974; Griffin et al 1975; Hesley et al 1976; Rabinowitz et al 1977; Chamberlain et al 1978; Schlegel and Kufner 1979; Ahlgren et al 1980; De Silva 1981; Gross 1981; Lynam and Nelson 1982; O´Flaherty et al 1982; Kang et al 1983; Campbell et al 1984).

Impact of skeletal lead upon blood lead level

The blood-lead level is, of course, affected by recent exposure. Further, blood-lead after end of exposure may be affected by absorption of lead remaining in the lungs and in the gastro-intestinal tract. In human radiotracer experiments, the absorption has usually been completed within 24 h (Hursh and Suomela 1968; Booker at 1969; Hursh and Mercer 1970; Morrow et al 1980; Chamberlain 1985). This is in favor of a limited effect of these unabsorbed pools on the blood-lead level, even during the initial period after end of exposure.

Such a rapid absorption is in accordance with the lack of accumulation as regards pulmonary lead content in deceased lead workers, in comparison with subjects without occupational

exposure (Barry 1975). On the other hand, increased levels of lead has been found in dead lead workers, who had, however, been exposed to a lead compound with low solubility (lead sulfide; Brune et al 1980; Gerhardsson et al 1985 and in press). But the amount of lead in the lung is rather small, even in lead workers only a few milligrams.

The subjects in the present study were probably mostly exposed to fairly soluble lead oxide. The effect of the lung lead pool on B-Pb should thus be limited, and cannot have had but a small impact on the considerably increased lead levels in blood a long time after end of exposure. Some absorption of lead earlier deposited in the lung may have caused a retardation of the elimination rate. However, this effect is probably small. Thus, the only possible explanation of the slow compartment seen in the elimination pattern in blood is that it reflects the large skeletal pool (**III**; **V**;Barry 1975; Brune et al 1980; Skerfving et al 1983). The association between finger bone-Pb and the slow compartment (Y(2)) of the B-Pb decay curve (**II**) strongly supports this

The release of lead from the skeleton into the blood stream (Blanchard et al 1969; Eisenbud et al 1969; Gotchy and Schiager 1969), causes an endo- genous lead exposure, which is dependent upon the size of the skeletal lead pool (**II**; **III**; **IV**; **V**), and which may be both considerable and long-lasting, as also indicated by some earlier studies (Prerovska and Teisinger 1970; Grandjean and Kon 1981; Hesley and Wimbish 1981; Mindus and Kolmodin- Hedman 1981; Neri et al 1983).

In a group of lead workers, the impact of the slow compartment (mainly the skeleton) on B-Pb (Y(2)), corresponded to as much as an average of 1.8 µmol/l, about 65% of the total blood lead level, and it ranged as far as up to 2.7 µmol/l (**II**). The impact of the endogenous release of lead was also clearly seen in the study of the relationship between B-Pb and finger bone-Pb in retired workers (**III**). In the ex-lead workers with the highest lead levels in finger-bone, B-Pb was elevated by about 1.5 µmol/l above the back-ground level.

The relative impact on B-Pb of the skeletal lead pool is, of course, also dependent upon the rate of recent absorption. The Y(2) fraction was thus usually higher in retired workers than in active ones (**II**). However, the active workers in our study were not representative for the average lead worker, since they were removed from exposure, due to high B-Pb, caused by heavy recent exposure, and had thus an unusually large lead pool in the fast compartment. In the retired workers, on the other hand, the exposure was probably gradually diminishing during some time prior to retirement, which had caused a reduction of the fast compartment. Also, the possible existence of an intermediately fast compartment would, in our study, probably have worked in favor of increasing the fraction of the slow compartment. In subjects without occupational lead exposure, fractions of B-Pb of10-70% have been estimated to be due to the skeletal lead (Manton 1977; Holtzman 1978; Chamberlain 1985; Manton 1985).

As mentioned above (**Introduction**), there is a nonlinear relationship between B-Pb and lead exposure, at least at relatively high exposures. Such a phenomenon might also exist as regards the endogenous exposure from the skeleton; in the active lead workers, the relative increment of Y(2) became less when the lead level in finger-bone increased (**II,** figure 6). An additional explanation of this possible nonlinearity is the different turn over rates in trabecular and cortical bone. Y(2), which represents the slow B-Pb decay phase, is, probably also associated with vertebra bone-Pb, although no significant correlation was obtained in the small material in our study. Between vertebra bone-Pb and finger bone-Pb would, theoretically, a curvi-linear relation be expected, since the accumulation rate in the two types of bone is different. However, no such a curvi-linear relation was obvious in the present material (**V**). Indeed, there was a good, and evidently rectilinear, association between B-Pb, which in the retired workers mainly represents Y(2), and finger bone-Pb

(III), as well as with vertebra bone-Pb (V), up to high bone-Pb levels.

Lead level in the skeleton of lead workers

The present data on lead levels in subjects without occupational lead exposure (III; V) is in accordance with earlier findings in in contemporary Scandinavians (Grandjean and Holma 1973; Grandjean 1975). It is higher than in prehistoric subjects, living in a world without traffic and industries (Ericson et al 1979), but lower than in contemporary subjects from the U.K. (Crawford and Crawford 1969; Barry 1975) and the U.S. (Kehoe 1961; Ericson et al 1979; Gross et al 1975; ICRP 1980).

The lead workers had much higher levels. The findings (III; IV; V) are agreement with some earlier observations in lead workers (Westerman et al 1965; Gossman and Heilenz 1967; Campbell et al 1970; Grandjean and Holma 1973; Barry 1975; Lindh et al 1978 and1980; Ahlgren et al 1980; Kijewski and Lowitz 1982; Skerfving et al 1983; Somervaille et al 1985; Wielopolski et al 1986), and in subjects with high lead exposure from other sources (Emmerson 1960; Eastwell et al 1983; Price et al 1984), including persons who died with lead poisoning (Vogt 1930; McKhann and Vogt 1933; Emmerson and Thiele 1960; Schwerd 1960; Kehoe 1961; Emmerson 1963; Gossman and Heilenz 1967). The skeletal lead content in lead workers may be in the order of magnitude of one gram.

As mentioned above, the present data (V) indicate a difference in lead levels in vertebra and finger-bone in most subjects. This is in accordance with some earlier observations (Barry 1975; Rabinowitz et al 1977; Skerfving et al 1983), and indicates the presence of at least two lead pools in the skeleton, one represented by the mainly trabecular vertebra and one by the mainly cortical finger-bone.

Kinetics of lead turnover in the skeleton

The data on the slow compartment in the blood lead decay curve is in favour of a median over-all half-time for the skeleton, during the time period studied, of about half a decade (II).

The half-life of lead in finger-bone (mainly cortical bone) was also about half a decade (IV). This is in accordance with the data on accumulation of lead in finger-bone after onset of occupational exposure (III), which indicates an elimination constant corresponding to a half-time of similar length. Even if the latter figure, due to redeposition of lead into the skeleton, is not exactly comparable to the actually observed elimination patterns, they still support each other.

In vertebra, the turnover is probably faster, as shown by the accumulation pattern after onset of occupational exposure, as well as by the different median ratios between lead levels in finger-bone and vertebra in active and retired lead workers (V). The half-time is probably a couple of years. The lack of association between individual ratios and time of exposure, and time after end of exposure, may be attributed to differences in exposure patterns and to differences in inter-individual turnover rates, as well as to analytical method errors.

Further, the existence of a close association between the lead level in vertebra and the half-time of the fast blood lead compartment, as well as with present B-Pb (V), and with chelatable lead (VI), clearly indicates that the lead in vertebra has a more rapid turnover and is thus better associated with the soft tissue lead pool and the present exposure, than is lead in finger-bone, where no associations, except with B-Pb in retired workers, were obtained

(III; V). Also, significantly higher ratios between lead level in finger-bone and vertebra in active than in retired workers, indicate a considerably faster turnover of lead in vertebra than in finger-bone.

The suggested turnover of the skeletal lead pool fits with some other observations, based on indirect methods or on limited data, by use of a metabolic model (Tsuchiya and Sugita 1971), data on radiolead excretion in urine (Muth and Oberhausen 1964; Khandekar et al 1972), decrease of radiolead measured *in vivo* in the skull in workers exposed to radium, radon, and radon daughters (Wrenn et al 1972; Cohen et al 1973), and on decay lead levels in bone biopsies (Westerman et al 1965; Gossman and Heilenz 1967) and in blood (Araki et al 1983).

But the presently proposed half-time is faster than has earlier been esti- mated (half-times up to 70 y) on basis of bone remodeling (Jaworowski 1965), various metabolic models (Petrow 1968; Fisher 1969; Holtzman 1970 and 1978; Bernard 1977; Smith and Hursh 1977; Sugita 1978; Steenhout 1982), and *in vivo* measurements of the decrease of radiolead in the skull (Cohen and Spitz 1975).

The differences may partly be due to the different methods and the varying turnover rates in the different parts of the skeleton studied. Also, the age of the groups studied may have had an influence, as osteoporosis, which occurs towards end of life, may affect the elimination rate. Moreover, and probably as important, it may depend on the time period studied, and thus the relative impact of different skeletal lead pools. In the present study, due to the limited time period studied, the existence of a rather fast skeletal compartment should be important, in some other investigations, slower compartments may have had a greater impact. A prolonged follow-up of the present subjects, with respect to lead levels in blood and skeleton, may allow a direct observation of such phenomena.

It may be mentioned in this connection, that Hodge et al (1970) found a half-time of fluoride of eight years in urine of subjects with high fluoride load in their skeletons . The half-time of fluoride in the skeleton thus seems to be similar to that of lead. Further, some data may indicate that the turn- over of lead is faster than that of strontium (Rivera 1965; Papworth and Vennart 1984). Also, it may differ from that of calcium (Heard and Chamberlain 1984). This may be due to an echange with non-active remodeling units in the bone.

Inter-individual variation in lead kinetics

Data on the elimination of the fast compartment of lead from blood after cessation of exposure indicate an inter-individual variation (II), which exceeds the uncertainty of the estimates of the true slopes. This is in accordance with the considerable inter-individual variations in metabolism, that have been noted as regards absorption of lead from the gut (Hursh and Suomela 1968; Blake 1976), distribution of lead in the erythrocytes (Raghavan et al 1980), turnover between different compartments in man (Marcus 1985a), and excretion of lead in the urine (Skerfving et al 1985).

Also, the data on the elimination of lead from blood during a long time after cessation of exposure, indicate an inter-individual variation in skeletal lead kinetics (II). Data in support of such a difference has also been reported in dogs (Fisher 1969).

The inter-individual variation in kinetics of lead metabolism, both in soft tissues and in bone, means probably a considerable difference between individuals as regards soft tissue and skeletal lead levels at a particular rate of absorption, and an accordingly different risk of

adverse effects. A rapid decrease of the fast compartment probably means a rapid elimination of lead from the body, which, of course, is an advantage. On the contrary, a rapid decrease of the slow compartment must be a result of a fast mobilization of substantial amounts of lead from the skeleton, which gives a high endogenous lead exposure, and which is, of course, a disadvantage.

Practical implications of the metabolic pattern

According to the present ordinance from the Swedish National Board of Safety and Health (1984), lead workers shall be removed from exposure when the B-Pb exceeds 3.0 μmol/l in one single sample, or 2.5 μmol/l in three consecutive determinations performed at three month´s intervals. The "return level" to lead work is 2.0 μmol/l. Similar levels are employed in several other countries.

If our typical lead worker, who had an Y(2) of 1.8 μmol/l, plus a back-ground level of 0.3 μmol/l, and a T1/2(1) of one month, is removed at the 3.0 μmol/l level, the removal has to last for about half a year before the 2.0 μmol/l level is reached. If the Y(2) is even more increased, to e. g. 2.2 μmol/l, the removal has to last for at least two years. A newly emloyed worker, however, who has a small Y(2), will display the same decay in less than one month. These teoretical values are supported by the actual observations in temporarily removed workers in our study (**II**; **Results**). In nine out of 19 cases of removal, the median Y(2) was 1.6 μmol/l and the median time 97 d untill the 2.0 μmol/l level was reached. In the rest of the cases, the median Y(2) was 2.2 μmol/l, and the "return level" was not reached even during a median period of removal of 180 d.

Skeletal lead as an index of past exposure

Some of the health effects of lead may be suspected to be induced by long-term exposure, or they may be irreversible, with clinical signs remaining for a long time, sometimes even after the exposure has been reduced and the B-Pb decreased significantly. This may cause problems as regards interpretation of the results in cross-sectional studies, in which present B-Pbs are correlated with measures of effect. In these cases, the skeletal lead level may give information, which allows conclusions regarding the earlier history of lead absorption in the individual.

In particular cases, the skeletal lead level has been estimated in bone biopsies (**V**; Westerman et al 1965; Gossman and Heilenz 1967; Rabinowitz et al 1977). But this is, of course, not a practical possibility for biological monitoring.

Measurements of the lead content of bone carried out *in vivo* by X-ray fluorescence technique is, of course, much better. The measurements have usually been performed in finger-bone (**III**; **IV**; Ahlgren at al 1976 and 1980; Eastwell et al 1983; Price et al 1984), but may also be made in tibia (Somervaille et al 1985; Wielopolski et al 1986). The measurements take about half an hour and causes only a low radiation dose. The detection limit in finger-bone is about 20 μg/g, which is much higher than the levels found in subjects without particular exposure (**III**; **V**; Grandjean and Holma 1973; Barry 1975; Grandjean 1975), but sufficient for use in the occupational setting (**III**; **IV**; Ahlgren et al 1976 and 1980), and in other particular exposure (Eastwell et al 1983; Price et al 1984). At measurements in the tibia, the detection limit is similar (Somervaille et al 1985). The method error at measurements in finger-bone is about 15% (**III**), which is sufficient for many purposes.

In lead workers, there is an increase of skeletal lead levels with increasing time of exposure (**III**; **V**), although the inter-individual variation in bone-lead concentration at a particular exposure time is considerable. That variation is, at least to a great extent, dependent upon variations in intensity of exposure, and accordingly, in lead absorption, in different individuals occupied in different working environments.

The present data show that there was a reasonable association between the time-integrated blood lead level and the lead level in finger-bone (**III**). An even better correlation (r_S=0.93 *vs* . 0.41) was seen in vertebra (**V**). The relationship should perhaps not be expected to be linear, since, as mentioned above, the relationship between exposure and blood lead level is non-linear. But the present data strongly suggests the possibilty to use lead levels in finger-bone as an index of exposure in cross-sectional studies of the relation between lead absorption and effect/response.

As mentioned several times earlier, the rate of turnover of lead in the finger-bone corresponds to a half-time of half a decade (**IV**). This means that, in this species of bone, a steady state is reached after a couple of decades of exposure (**III**; Skerfving et al, in press). The finger-bone lead level can thus be expected to give a picture of the time-integrated lead absorption during a decade back. It is possible, that use of a more typical cortical bone, such as the tibia (Somervaille et al 1985), may give a picture of the lead exposure even further back in time. But that possibility remains to be investigated.

In a similar way, the lead level in teeth has been used as an index of lead exposure, especially in shedded deciduous teeth in children (e.g. Needleman et al 1979; Möller et al 1982). But methods have also been developed for determination of lead in teeth *in situ* (Bloch et al 1976).

Chelatable lead, no index of skeletal lead

The exponential association between mobilized, as well as unmobilized, urinary lead excretion and the blood lead level, suggests, that the excretion is associated with the lead level in plasma, which is known to be exponentially associated with B-Pb (De Silva 1981; Manton and Cook 1984).

The excretion of mobilized lead was the same in the active workers as in the retired ones at a particular B-Pb. This indicates, that the per-oral administration of penicillamine was not associated with any important increase of the gastro-intestinal absorption of lead, which was probably swalloved by the active workers during and after work.

In active, as well as in retired workers, there was a close correlation between the excretion of mobilized lead and vertebra bone-Pb (**VI**). The considerably higher (about three times) excretion in the active workers than in the retired ones at a particular vertebra bone-Pb, reflects, most probably, a higher lead concentration in the soft tissues. The active workers suffered, contrary to the retired ones, from an ongoing exogenous exposure in addition to the endogenous exposure from the skeleton. A higher lead concentration in the soft tissues of active workers, as compared to the retired ones, is also strongly indicated by the fact, that the B-Pb was higher in active workers than in retired ones with the same vertebra bone-Pb (**V**).

The present data (**VI**) indicate that lead excretion in the urine during penicillamine mobilization primarily reflects the soft tissue pool of lead, which in turn is in a dynamic equilibrium with the lead pool in the trabecular bone (vertebra). The normal, unmobilized

lead excretion in urine, is probably as good an index of the soft tissue lead pool, as is the mobilized lead. Analytically, however, it is somewhat more difficult to quantify, since the levels are lower.

Neither mobilized or unmobilized urinary lead reflects the lead level in the finger-bone, which is mainly cortical. Wielopolski et al (1986) found an association between EDTA-chelatable lead and lead levels in the surface bone of the tibia, which is probably closer associated with the lead in vertebra than with lead in massive, cortical bone. The results may thus be in accordance with the results of the present study (**VI**). However, the chelation test does not indicate the lead level in finger-bone, and, most probably, neither in the deeper parts of any other cortical bone, which contains the major lead pool in the body. Thus, chelatation tests cannot substitute measurements of lead in cortical bone as an index of the amount of lead accumulated in an individual.

CONCLUSIONS

1. The decay of blood lead after end of exposure may well be described by a two-compartment exponential model, with average half-times of about 1 month and about 5 y for the fast and the slow compartments, respectively.

2. The half-times of the compartments of the blood lead decay curve show significant inter-individual variations, probably indicating a difference in lead metabolism, and thus a difference in risk between individuals at the same exposure level.

3. In lead workers, lead accumulates in the skeleton. The average lead level in lead workers was more than twenty times higher than in occupationally non-exposed subjects; levels up to about 150 μg/g were found.

4. The lead pool in the skeleton affects the blood lead level, even many years after end of exposure. This is the main origin of the second compartment of the blood lead decay curve. A high skeletal lead level (about 100 μg/g), causes an average increase in the blood lead level by about 1.5 μmol/l.

5. In individuals, there are differences in lead levels in finger-bone and vertebral bone. This means that there are at least two compartments of lead in the skeleton, one in cortical (reflected by finger-bone) and one in trabecular bone (reflected by vertebra). The kinetics of lead in the two types of bone is different.

6. The mean half-time for the decay of lead after end of exposure in finger phalanx was estimated at about 6-7 y. The half-time for lead in vertebra is shorter, probably 1-2 y.

7. The above- mentioned significant inter-individual difference in the half-time of the slow blood lead compartment, which most probably reflects the skeletal lead pool, indicates possible differences in the kinetics of the skeletal lead.

8. The lead level in finger-bone, as well as in vertebral bone, reflects the time and intensity of lead absorption. *In vivo* measurement of lead in finger-bone offers a tool for determination of the accumulation rate of lead in finger-bone in lead workers, at least during the first decade of exposure.

9. The urinary excretion of lead during a mobilization test with penicillamine is associated with the lead level in vertebra, but does not indicate the lead level in finger-bone, and is thus probably no index of the cortical lead pool in the skeleton, which is often the major part of the body burden.

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PAPERS I - VI:

I. Schütz, A. and Skerfving, S., 1976, Effect of a short, heavy exposure to lead dust upon blood lead level, erythrocyte delta-aminolevulinic acid dehydratase activity and urinary excretion of lead, delta-aminolevulinic acid, and coproporphyrine, Scand. J. Work Environ Hlth 3:176-84.

II. Schütz, A., Skerfving, S., Ranstam, J., and Christoffersson, J.O., Kinetics of lead in blood after end of occupational exposure. Submitted for publication.

III. Christoffersson, J.O., Schütz, A., Ahlgren, L., Haeger-Aronsen, B., Mattsson, S., and Skerfving, S., 1984, Lead in finger-bone analysed *in vivo* in active and retired lead workers, Am. J. Ind. Med. 6:447-457.

IV. Christoffersson, J.O., Schütz, A., Skerfving, S., Ahlgren, L., and Mattson, S., In press, Decrease of skeletal lead levels in man after end of occupational exposure, Arch. Environ. Hlth.

V. Schütz, A., Skerfving, S., Christoffersson, J.O., Ahlgren, L., and Mattson, S., Lead in vertebral bone biopsies from active and retired lead workers. Submitted for publication.

VI. Schütz, A., Skerfving, S., Christoffersson, J.O., and Tell, I., In press, Chelatable lead vs. lead in human trabecular and compact bone. Sci. Tot. Environ.

Scand. j. work environ. & health 3 (1976) 176—184

Effect of a short, heavy exposure to lead dust upon blood lead level, erythrocyte δ-aminolevulinic acid dehydratase activity and urinary excretion of lead, δ-aminolevulinic acid and coproporphyrin

Results of a 6-month follow-up of two male subjects[1]

by ANDREJS SCHÜTZ, B.Sc., and STAFFAN SKERFVING, M.D.[2]

SCHÜTZ, A. and SKERFVING, S. Effect of a short heavy exposure to lead dust upon blood lead level, erythrocyte δ-aminolevulinic acid dehydratase activity and urinary excretion of lead, δ-aminolevulinic acid and coproporphyrin: Results of a 6-month follow-up of two male subjects. *Scand. j. work environ. & health* 3 (1976) 176—184. During 1 h two healthy volunteers, not earlier exposed occupationally to lead, inhaled about 100 mg of lead as a mixture of lead oxides and lead sulfate. Maximum blood lead (PbB) concentrations of about 0.5 mg/l and minimum blood cell δ-aminolevulinic acid dehydratase activities (ALAD) (6 % of the preexposure values) were observed within 38 h after exposure. PbB and ALAD returned to preexposure levels after about 300 and 150 days. A highly significant correlation between ALAD and PbB was seen even at lead levels in the range 0.1—0.2 mg/l. Delta-aminolevulinic acid, coproporphyrin and lead in the urine (ALAU, CPU, and PbU, respectively) increased. The peak levels occurred after about 15 h for ALAU and CPU and after about 24 h for PbU. There was a very good correlation between log PbU and lin PbB. ALAU increased already at PbB levels of about 0.3 mg/l.

Key words: lead, experimental exposure, excretion, blood, urine, δ-aminolevulinic acid, δ-aminolevulinic acid dehydratase, coproporphyrin, half-time.

Workers under surveillance because of lead (Pb) exposure sometimes show unexpected, temporary rises in their blood lead (PbB) levels. A possible explanation could be the occurrence of a short, but intensive, exposure in addition to continuous occupational exposure and "background" nonoccupational exposure. In blood samples obtained from workers before and after 8-h workdays in workroom levels of 0.2 mg Pb/m^3 or less, we found no significant PbB rise. Thus we conducted an experiment in which persons formerly exposed only to nonoccupational "background" lead were subjected to lead levels higher than 0.2 mg Pb/m^3. Such an experiment also provided the opportunity to study lead metabolism,

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information on which is scanty in several aspects (7, 9, 31, 36). It should also supply intraindividual data from humans on the relationship between lead levels in blood and urine on one hand and different biochemical parameters on the other. This relationship has usually been studied only in exposed groups, and interindividual variation has affected the results.

MATERIAL AND METHODS

Subjects

Two male volunteers 33 and 37 years of age participated in the investigation. They had no earlier occupational exposure to lead. Subject 1 was a smoker (about 10 cigarettes/day) and subject 2 a nonsmoker. Their weights were 78 and 80 kg. Both were healthy. They had normal hemoglobin levels in blood, normal creatinine levels in serum, no glucosuria or albuminuria, a normal ability to concentrate urine, and normal liver function as indicated by serum activities of aminotransferases (S-ALAT and S-ASAT).

Exposure and sampling

The volunteers were exposed for 60 min to lead dust (a mixture of lead oxides and lead sulfate) in a closed section of a destruction facility for lead storage batteries. Samples for the determination of lead in air were obtained during exposure, those for the determination of PbB (whole blood) and δ-aminolevulinic acid dehydratase activity (ALAD; EC 4. 2. 1. 24) in red blood cells (RBC) were obtained on several occasions before exposure and during a period of 500 days after it, and those for the determination of lead, δ-aminolevulinic acid, and coproporphyrins in urine (PbU, ALAU, and CPU, respectively) were collected during the postexposure period.

Lead in air. We determined individual exposure from dust samples collected on cellulose acetate membrane filters (Millipore; pore diameter 0.8 μm) with a portable pump (MSA model G; 2 l/min) in the breathing zones of the subjects. The filters were treated with concentrated nitric acid. After dilution of the samples with deionized water, the lead content was determined by atomic absorption spectrophotometry (AAS). The detection limit was 2.5 μg, which corresponds to an air level of 25 $\mu g/m^3$. The combined method error, including the determination of air sample volume and lead content, was calculated to be 7 %. The average lead concentration in the breathing zones of the two subjects (data on subject 1 will always be presented first) was 51 and 54 mg/m^3. Lung ventilation, as estimated under the same conditions on a later occasion with the use of Douglas bags, was about 30 l/min. The inhaled amount of lead was thus calculated to be 90—100 mg for the two subjects.

Particle size of lead dust. Dust for the determination of particle size was sampled on cellulose acetate membrane filters (Millipore; pore size 0.22 μm) and on Nuclepore membrane filters (General Electric; pore size 0.4 μm). The acetate membrane filters were made transparent by treatment with acetone vapor, and the particle size was estimated by bright field microscopy at a magnification of 400 times. The particle size distribution on the Nuclepore filters was determined in photographs taken at a magnification of 1,200 times in a scanning electron microscope. Of the particles 93 % were in the range 0.5—5 μm (table 1).

Dust samples were also collected with a gravimetric size-selecting personal dust sampler (C. F. Casella & Co., LTD) (1). During two 30-min sampling periods 9 and 11 % (w/w) of the lead passed the cyclone size-selector.

Lead in blood. Venous blood samples were obtained in heparinized acidwashed tubes. On each occasion two to four 5-ml samples of blood were wet-ashed with nitric acid, perchloric acid, and sulfuric acid. The lead was extracted as a dithizone complex in methyl isobutyl ketone and determined by AAS. A satisfactory accuracy was ascertained by repeated interlaboratory checks with independent methods (AAS and spectrography). The limit of detection was 0.01 mg/l, and the method error, as calculated from 20 duplicate determinations in the concentration interval 0.04—0.12 mg/l, was 4 % of the mean.

Table 1. Particle size distribution for the aerosol of lead oxides and lead sulfate inhaled by the volunteers.

Particle size (μm)	Percentage distribution	
	Bright field microscope [a] (x 400)	Scanning electron microscope [b] (x 1,200)
< 0.5	— [c]	— [c]
0.5 — 1	57	25
1 — 5	35	68
5 — 10	6	6
10 — 15	1	1
> 15	<< 1	< 1
Total	99 +	100 +

[a] A total of 1,090 particles were counted.
[b] A total of 206 particles were counted.
[c] Neither method permitted the counting of particles smaller than 0.5 μm. With scanning electron microscopy using a magnification of x 6,000, only a few particles smaller than 0.5 μm were detected.

Lead in urine. During the first 3 days after exposure all urine was taken from each voiding. Later 24-h urine samples were collected during at least three consecutive days. PbU, ALAU, CPU, and creatinine levels were all determined from these samples.

PbU was determined spectrophotometrically after the lead was precipitated (coprecipitation with calcium from 40 ml of urine added to ammonia), the precipitate was dissolved in diluted hydrochloric acid, and the lead dithizonate was extracted in chloroform after the addition of a citric acid ammonia cyanide buffer (pH 11—12). The method is a modification of the procedure described by Amdur (2). The main modification involves adding copper sulfate and nitric acid to the sample and heating it to 90° C for 1 h before the precipitation reaction; in this manner lead recovery is increased in different samples from 30—90 % to 100 %. Each sample was analyzed twice. The results obtained with this procedure agreed well with those determined with independent AAS (after wet-ashing) and colorimetric methods. The detection limit was about 0.003 mg/l. The method error, as calculated from 20 duplicate analyses in each interval of 0.005—0.020, 0.02—0.05, 0.05—0.10, and 0.1—0.2 mg/l was 14, 6, 4 and 3 %, respectively.

ALAD activity in blood. Determinations of ALAD activity in blood were performed within 1 h after sampling according to the method of Bonsignore et al. (5) with some modifications (28). The method error, as determined by analyses of duplicate samples within the interval 60—200 μmol porphobilinogen (PBG) $\cdot$ h^{-1} $\cdot$ 1 RBC^{-1}, was 5 % at the low end of the interval and 3 % at the high end. Methylmercury (28) and ethanol (19) causes a decrease in ALAD activity. Both subjects had "normal" mercury levels in their blood cells (below 10 μg/kg). No intake of alcohol occurred during 24 h before the sampling.

ALA in urine. Determinations of ALAU were made according to Grabecki et al. (10). They were corrected with regard to the variation in the recovery of ALA in urine samples with different creatinine concentrations with the use of the function $y = 133 - 21\ x$ $(0.5 < x < 2.5;\ n = 10;\ r = -0.96)$, where y denotes recovery in per cent and x the creatinine level in grams per liter. The detection limit was about 1 mg/l. The method error, as calculated from 20 duplicate analyses in the range 5—10 mg/l, was 7 %.

Coproporphyrin in urine. The CPU was determined according to Askevold (3). Coproporphyrinogen was oxidized by the method of Schwartz et al. (27). The calculations were made according to Rimington and Sveinsson (24) and With (38). The detection limit was about 0.01 mg/l, and the method error, as calculated from 20 duplicate samples in the range 0.10—0.20 mg/l, was 3 %.

Creatinine in urine. Creatinine was determined in all samples according to the method of Teger-Nilsson (32). The method error of 10 consecutive analyses of one sample was 2 %.

Calculations of PbB half-times

Data on PbB versus time was fitted to the sum of two exponential functions with the weighted least squares method. The analytical error was assumed to be proportional to the measured values.

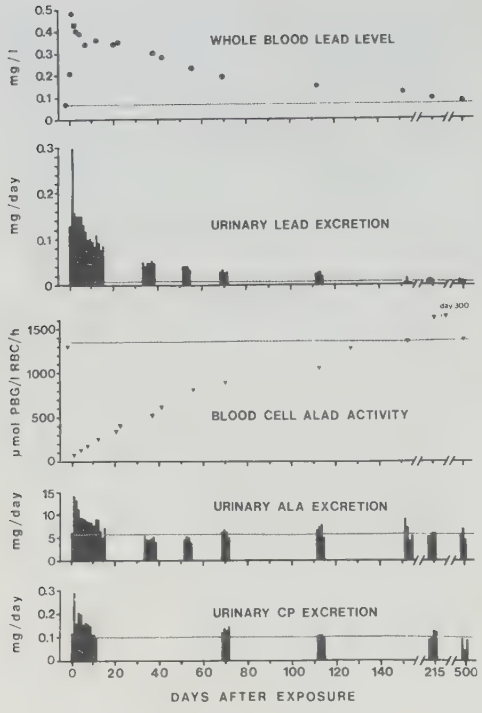

Fig. 1. Levels of lead in whole blood and in urine, δ-aminolevulinic acid dehydratase (ALAD) activity in blood cells, levels of δ-aminolevulinic acid (ALA) and coproporphyrin (CP) in urine before and after a short, heavy inhalation exposure of subject 2 to lead. The dotted lines indicate "background" levels present before exposure and after the end of decay.

RESULTS

The pattern of PbB, PbU, ALAD, ALAU, and CPU was similar in both subjects. One set of data is presented in fig. 1.

Lead in blood

Before exposure the PbB of both subjects was about 0.08 mg/l (fig. 1). In blood samples obtained about 10 min after the end of exposure PbB was 0.16 and 0.21 mg/l. The next morning, 14 h after exposure, the highest levels, 0.44 and 0.48 mg/l, were measured; they were about six times higher than the "background" level. After an additional 24 h the levels had decreased to 0.37 and 0.43 mg/l, and after about 250 and 300 days they had returned to levels practically identical with the pre-exposure ones.

The decrease in PbB was initially very rapid, but slower later on (fig. 2). When the decay curves were fitted to the sum of two exponential functions, the fast ones had half-times of 1.3 and 0.5 (95 % confidence limits 0.7—5 and 0.2—∞) days, and the slow ones half-times of 46 and 49 (confidence limits 41—52 and 45—54) days.

Lead in urine

PbU rapidly increased to peak levels that were about 50 times higher than the "background" levels (17 and 19 µg/h; 240 and 210 µg/g creatinine) in samples voided about 24 h after exposure, and a slow decrease followed, the "backround" level being reached approximately 150 days later.

The total amount of lead in the urine in excess of the "background excretion" was

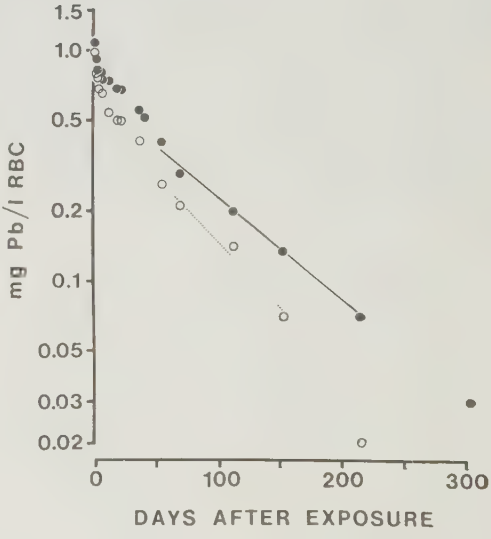

Fig. 2. Levels of lead in red blood cells (RBC) after a short, heavy inhalation exposure of subjects 1 (circles) and 2 (dots) to lead. "Background" levels have been subtracted and logarithms of the differences plotted.

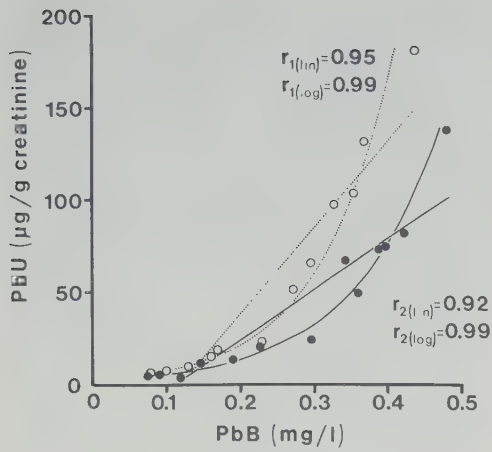

Fig. 3. Relation between levels of lead in whole blood (PbB) and the excretion of lead in urine (PbU) at different times after a short, heavy inhalation exposure of subjects 1 (circles) and 2 (dots) to lead. Both linear and exponential regression lines are indicated together with the corresponding coefficients of correlation.

calculated to be about 5 mg from the areas under the decay curves (fig. 1).

There was an obvious correlation between PbU and PbB (fig. 3). The data for each subject fitted a single exponential function ($r_1 = r_2 = 0.99$) better than a rectilinear one ($r_1 = 0.95$ and $r_2 = 0.92$).

ALAD activity

The impact of the absorbed lead on ALAD activity was very rapid. The minimum activities — in both subjects 6 % of the preexposure values — were measured in the first samples taken after exposure for ALAD activity analysis, i.e., 14 h after the end of exposure.

ALAD activity recovered as PbB decreased, and after 200 to 300 days it even exceeded the preexposure levels in both subjects. In samples analyzed 500 days after exposure the activities had returned to preexposure values.

Excellent intraindividual correlations were found between ALAD activity and lead in the RBC (fig. 4). The data fitted single exponential curves somewhat better than rectilinear ones ($r_1 = r_2 = -0.97$ for log ALAD; $r_1 = -0.95$ and $r_2 = -0.97$ for lin ALAD). A statistically significant correlation ($r_1 = -0.86$, $p < 0.02$ and $r_2 = -0.92$, $p < 0.01$) existed already at the PbB interval of 0.1—0.2 mg/l.

ALA in urine

ALAU was increased above the "background" level (0.3 and 0.2 mg/h; 4 and 3 mg/g creatinine) in subject 1 for about 1 week and in subject 2 for about 2 weeks after exposure. A maximum excretion of three times higher than the "background" (0.7 and 0.7 mg/h; 11 and 9 mg/g creatinine) occurred in samples voided about 15 h after exposure, — i.e., simultaneously with the highest PbB level observed but nearly 10 h earlier than the maximum excretion of PbU.

A clear increase in ALAU was seen above a PbB level of 0.3 mg/l (fig. 5). A statistically significant correlation was obtained for one of the subjects ($r_2 = 0.95$, $p < 0.01$) at PbB levels above 0.3 mg/l.

Coproporphyrin in urine

The pattern of coproporphyrin excretion was similar to that of ALA — the maximum excretion occurred about 15 h after exposure and was about three times higher than the "background."

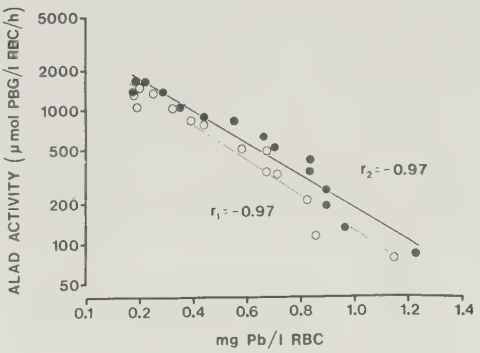

Fig 4. Relation between lead and δ-aminolevulinic acid dehydratase (ALAD) activity in red blood cells (RBC) at different times after a short, heavy inhalation exposure of subjects 1 (circles) and 2 (dots) to lead. Logarithms of ALAD activity have been plotted.

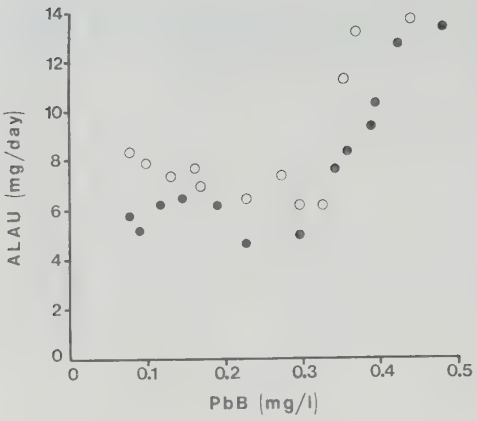

Fig. 5. Relation between levels of lead in whole blood (PbB) and the 24-h excretion of δ-aminolevulinic acid in urine (ALAU) at different times after a short, heavy inhalation exposure of subjects 1 (circles) and 2 (dots) to lead.

DISCUSSION

The metabolism of lead is complicated. Models containing from two (30) to four (20) compartments have been proposed. Rabinowitz et al. (22) fitted data on one human, subject only to "background" exposure, with a three-compartment model. The first two pools (blood and other soft tissues?) had half-times of about 20 days. The authors also found one compartment (bone?) which had a half-time of 20 years. A very slow decay of the body burden may also be anticipated from PbB data on "non-exposed" former lead workers (13, 21).

One may assume that the decay in PbB (fig. 2) in the present investigation reflects the excretion kinetics of two compartments with different half-times (blood and soft tissues?). Thus we fitted the curves with the sum of two exponential functions. However, the following three objections may be raised to this assumption: (a) The shape of the initial part of the curve may have been affected by blood absorbing lead from the lungs and the gastrointestinal tract simultaneously as lead was cleared from the blood into other tissues and urine. The rising PbB levels indicated that an absorption of lead occurred already during the 1-h exposure period. The PbB started to decline already within 24 h after the end of exposure; therefore the major part of the absorption was probably completed within that short period. This result agrees with earlier findings (17). (b) Only for subject 1 could the presence of two different functions be firmly established. For subject 2 the range of the 95 % confidence limits of the half-times of the two functions overlapped. (c) The curvilinear relation obtained between PbU and PbB indicates that the urinary clearance of lead from whole blood may not follow first-order kinetics at high PbB levels, at least in recently exposed subjects. At levels of 0.3 mg Pb/l RBC (PbB 0.14 mg/l, "background" level subtracted) and lower, the decay curves fit well with single exponential functions and the decay rates are remarkably similar for the two subjects. At these levels the relation between PbU and PbB is reasonably rectilinear (fig. 3). It may be assumed that this part of the curve mainly reflects clearance from a fairly rapid compartment (soft tissues?).

The estimated total amount of lead inhaled by the volunteers was 90—100 mg. It is impossible to make a reliable estimate of the fraction deposited in and absorbed from the lungs. The rest was cleared from the respiratory tract, and most of it was swallowed and partly absorbed from the gastrointestinal tract. An assumption of 5—10 % absorption in the lung (as judged from the fraction passing the particle size-selector) and 5—10 % of the rest in the intestine (23) would indicate an absorbed dose ranging from 10 to 20 mg. About 5 mg of the dose appeared in the urine during the study. Published data on the elimination of lead in feces is not consistent (8), but probably a similar amount or less (23) was excreted through that route. Thus it may be assumed that the dose was not completely eliminated during the study. The rest must have been retained in the body in a slow compartment, mainly in the bone. The lead content of the skeleton is — even in occupationally non-exposed subjects — relatively high (4, 26, 37) when it is compared to the amount that could possibly have been retained in this study. Thus, when the analytical method

errors are considered, no significant persistent elevation of PbB as a result of a small increase of lead in the skeleton should be expected after the lead contents of the rapid compartments have decayed.

The curvilinear relation between PbU and PbB has not been reported earlier, but our findings agree with the observations that poisoned subjects may daily excrete considerable amounts of lead in their urine (18). The shape of the curve may be due to (a) absorption of lead from the lungs and the gut and a subsequent relatively higher rise in plasma lead level than in RBC lead level (23) and/or (b) an increasing ratio between lead levels in plasma and blood cells (because of binding conditions) as whole blood levels rise (6); in both cases the renal glomerular filtration rate would be affected. Lead in urine and plasma may very well be linearly related. Also, theoretically, (c) a limited capacity of tubular reabsorption of lead could give a curvilinear relation. However, the limited data on hand (39) indicate a tubular reabsorption at high PbB levels only.

We found a dramatic decrease in ALAD activity in the RBC already within 14 h after the short heavy exposure, i.e., earlier by days than the decrease reported in workers at the onset of lead exposure (16, 35). It is reasonable to assume that ALAD activity decreased already during the exposure as PbB rose. The activity increased as soon as PbB decreased, i.e., within 24 h after the end of exposure. A recovery of activity has previously been observed in workers examined 4 months after the end of exposure (13, 33).

It is also noteworthy that, in accordance with observations on the relationship between ALAD and PbB in lead workers during and after exposure (13, 33), ALAD activity even exceeded the preexposure level during one period in our experiment. This result might have been due to a compensatory increase in ALAD (or any other biochemical function which optimizes ALAD activity) in the erythropoetic system and a subsequent enhanced activity in young RBC. Another possibility is the formation of a lead-inactivating compound.

There was an excellent intraindividual correlation between ALAD activity and PbB in our study, far better than has been reported for groups of individuals (11, 12, 13, 16, 25, 34) for whom interindividual variations influenced the results. There has been some discussion on whether a threshold PbB exists with regard to impact on ALAD activity. Some authors have claimed an effect already at PbB levels of 0.1—0.2 mg/l (14, 15, 34), while others (11, 12, 13, 25) could demonstrate effects only at higher PbB levels. The present data clearly show an effect at 0.1—0.2 mg/l, i.e., close to levels usually found in "nonexposed" Swedes (12).

A clear increase in ALAU was noted at PbB levels of 0.3 mg/l, which is lower than the 0.4 (29, 35) — 0.5 (12, 25, 34) mg/l reported in studies of groups of workers.

CPU seems to be as sensitive as ALAU in the determination of lead exposure. However, we did not measure the level in all urine specimens, and thus the threshold PbB resulting in elevated CPU cannot be given.

It is remarkable that the peaks of ALAU and CPU occurred about 10 h before the peak PbU. As the time of the peak PbB is not exactly known, the discrepancy cannot be elucidated. If the peaks of PbB, ALAU, and CPU occurred simultaneously, the PbU delay may be due to diurnal variation (18). If, however, the peak PbB occurred simultaneously with the peak PbU, a partial compensatory restitution of heme synthesis (as mentioned previously) might already have occurred.

The short heavy exposure of the volunteers caused a rapid and considerable increase in PbB. Although it must be kept in mind that the present data were obtained from two formerly "occupationally unexposed" subjects after inhalation of a very heavy single dose, it is reasonable to assume that occupationally exposed workers with elevated PbBs would show a further rise in PbB after a heavy additional dose. It is, however, not possible to predict the increase since the rise in PbB might depend upon the lead already present in different body compartments. Because of the apparent curvilinear relation between PbU and PbB, a rapid elimination of additional lead may be expected and thus a more rapid decrease in PbB to the level present before the additional exposure may occur.

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Kinetics of Lead in Blood after End of Occupational Exposure

II

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Abstract

The sum of two exponential functions was fitted to the decay of blood-lead level (B-Pb) after end of lead exposure. In two formerly occupationally non-exposed subjects, who had been exposed to a single short heavy dose, the fast compartment (probably soft tissues) had a biological half-life of 27 and 44 d, respectively. In 20 lead workers, studied after end of occupational exposure, the corresponding median was 29 (range 7-63) d. In 21 ex-lead workers, the median biological half-life of the slow compartment was 5.6 (range 2.3-27) y. There were significant inter-individual variation in both the fast and the slow half-life. This probably means a considerable variation in risk at a certain exposure level. In the lead workers, the fraction of B-Pb corresponding to the slow compartment (from extrapolation back to time of end of exposure) had a median as high as 1.8 (range 0.7-2.7) µmol/l, which constituted more than half of the total B-Pb. This fraction was associated with the exposure history, as well as with the lead level in the skeleton, the latter determined in vivo by an X-ray fluorescence method. The data thus indicates a rather rapid turnover of the skeletal lead pool, which may affect the B-Pb considerably.

Introduction

Lead exposure is common in the industry. The lead level in blood (B-Pb) is the main parameter used for biological monitoring of exposure (74). However, the knowledge of the kinetics of B-Pb is incomplete, as it is, regarding the metabolism of lead in the other tissues of the body (74), which B-Pb may mirror.

We here report a study of the B-Pb decay pattern in lead-exposed subjects after the end of exposure. This decay pattern could be interpreted by a metabolic model.

Study population and methods

Subjects and sampling

Twenty-three male ex-lead workers (table 1) were studied. Their mean age was 55 y and their mean exposure time 23 y. B-Pb was usually determined at end of exposure and then at varying intervals. In ten subjects B-Pb was determined once a year or more often. In one worker, no information is available concerning the first year after end of exposure. In 12 subjects, several determinations were made during the first year, then at year seven and then again from year nine on about twice a year. In one subject there is a lack of data between 3.5 and 9.6 years after end of exposure.

In addition, 17 male lead workers temporarily removed from exposure were investigated (table 2). Their mean age was 49 y and their exposure time 11 y. The reason for removal from exposure was high B-Pb (generally about 3.0 µmol/l or more). Twelve of the workers were transferred from a smelter to a nearby plant, the work in which did not cause lead exposure. B-Pb was generally determined at removal, and then once a week for three weeks, later on once every 2-4 weeks.

Further, two male formerly occupationally non-exposed subjects, who had been exposed to a single short heavy lead dose, were included (table 2). Details on these two subjects have been published elsewhere (56).

Spot determinations of "back-ground" B-Pb were made in 47 healthy workers not occupationally exposed to lead. They lived in the county and were all blue collar workers. Their average B-Pb was 0.3 µmol/l. In this connection, it may be mentioned, that in 15 workers, in a glue-production plant, located close to the non-lead resort of the temporarily removed smelter workers, the average B-Pb was 0.5 (range 0.3-0.7) µmol/l.

Medical examinations

In most subjects, an occupational and medical history, including alcohol habits, was obtained. Venous blood samples were analysed for (B-Pb, see below), hemoglobin, sedimentation rate, and red and white cell counts, as well as calcium, phosphate, and creatinine concentrations, and alkaline phosphatase (S-ALP) and gamma-glutamyl transferase (S-GT) activities in serum. A urine sample was analysed for albumine and glucose.

Among the ex-lead workers, detailed medical information is lacking in four. Among the 19 remaining, 12 had earlier been removed temporarily from lead exposure because of high B-Pb and/or U-ALA at least once. One subject was clinically lead poisoned (upper abdominal pain, constipation, neuropathy, and slight anemia) at the time of the end of exposure. Nobody else had been treated in hospital because of lead poisoning. One worker had a clinically silent chronic lymphatic leukemia and one had a type 2 diabetes treated with diet only. Three persons had slight increases of serum creatinine levels and two others slight albuminuria. Three subjects had somewhat increased S-GT, two of whom were known to abuse alcohol.

Among the 17 temporarily removed lead workers, detailed medical information was available in 14. Among those, seven had earlier been removed because of high B-Pb and/or U-ALA. Nobody had been treated with drugs because of lead poisoning. Three subjects had slightly increased S-GT, one of whom also S-ALP. One person had an isolated marginal increase of S-ALP.

The two subjects without previous occupational lead exposure were both in excellent health.

Blood-lead determinations

Blood was obtained from the cubital vein in acid-washed heparinized tubes.

B-Pb determinations in almost all samples were made in the same laboratory and by the same method. The samples were wet-ashed and lead was complexed with dithizone, extracted, and determined by flame atomic absorption spectrometry (AAS; 55, 56). The detection limit was 0.05 µmol/l (10 µg/l).

Each analytical series contained six samples, two blanks containing reagents only, and four "normal" blood samples, two of them with standard lead addition. All samples were analysed twice. The coefficients of variation, as calculated from duplicate analyses in 25 samples containing 0.5 µmol/l or less was 6.6 per cent of the mean, in 57 samples containing 0.5-1 µmol/l 3.9 per cent, in 58 samples containing 1-2 µmol/l 2.5 per cent, and in 60 samples containing 2-3.5 µmol/l 2.0 per cent.

Table 1. Kinetics of decrease of lead in blood (B-Pb) after end of occupational exposure in 23 ex-lead workers observed for more than one year.

Subject No.[1]	Age[2] (y)	Exposure time (y)	First B-Pb (μmol/l)	Observation time (y)	No. of samples	One compartment R^2[3] (%)	Two-compartment model: Fast compartment $T_{\frac{1}{2}}(1)$ (CI)[4] (d)	Fast compartment Y(1)[5] (μmol/l)	Slow compartment $T_{\frac{1}{2}}(2)$(CI)[4] (y)	Slow compartment Y(2)[5] (μmol/l)	Two-compartment model R^2[3] (%)	Three compartments R^2[3] (%)
101[6]	44	4.5	1.8	2.9	5	68	30[7]	0.0	3.7(2.0-27)	1.9	68	68
102	60	5	4.2	15.0	39	87	29(21-47)	1.4	8.4(7.6-9.5)	2.6	97	97
103	49	10	5.9	5.1	40	68	39(33-47)	3.0	4.2(3.4-5.7)	2.2	98	99
104	54	35	2.7	13.0	11	87	30[7]	1.4	2.3(0.9-oo)	0.7	95	95
105	41	3	3.0	12.9	10	88	30[7]	0.8	5.6(4.3-8.2)	1.9	94	94
106	48	8	2.9	12.9	12	91	30[7]	1.0	4.6(4.2-5.1)	1.6	99	99
107	54	34	3.3	13.0	13	94	30[7]	1.1	3.5(2.8-4.7)	2.0	98	99
108	30	7	3.7	12.9	12	91	30[7]	1.2	4.6(4.2-5.2)	2.2	99	100
109	59	27	2.6	12.9	12	79	30[7]	1.0	5.1(4.5-5.8)	1.2	99	99
110	56	26	3.8	12.9	12	51	30[7]	1.7	7.6(6.7-8.8)	1.8	99	100
111	65	45	3.3	12.8	12	86	30[7]	1.0	9.4(7.9-11.5)	2.0	97	98
112	51	33	3.2	12.8	10	95	30[7]	0.5	5.8(5.0-6.9)	2.3	98	99
113	66	44	3.0	11.2	9	96	30[7]	0.5	5.6(4.7-6.8)	2.3	98	100
114	31	4	2.4	10.4	8	98	30[7]	0.2	0.8(0.5-2.5)	2.0	98	99
115	63	45	2.2	9.4	6	96	30[7]	0.3	3.9(3.3-4.8)	1.6	99	99
116	67	10	4.3	13.2	9	82	30[7]	1.4	8.7(6.7-12.4)	2.5	97	97
116[6]	59	27	2.0	4.2	14	31	30[7]	0.1	18 (8.1-oo)	1.6	35	37
118	58	22	1.9	4.9	10	78	30[7]	0.4	6.5(4.3-13.8)	1.2	91	91
119	65	30	2.8	4.6	13	86	30[7]	0.8	4.7(4.0-5.8)	1.8	98	97
120	65	33	2.3	4.6	14	64	30[7]	0.6	9.5(6.2-21.3)	1.3	89	89
121	65	14	1.8	4.3	17	78	30[7]	0.4	4.3 (3.3-6.4)	1.0	92	92
122	61	24	1.8[8]	3.6	12	9	30[7]	4.8	oo (oo-oo)	1.4	0	13
123	65	38	2.0	1.7	12	27	7(2-oo)	0.3	27 (5.5-oo)	1.5	50	50

1) No. 101 was a cast bronze founder, No. 102 spray-painter, Nos. 103-115 storage battery workers, No. 116 wire lead coater, and Nos. 117-123 smelter workers.
2) At end of exposure.
3) R^2 is degree of explanation.
4) $T_{\frac{1}{2}}(1)$ and $T_{\frac{1}{2}}(2)$ are half-lives of first and second compartments, respectively. CI=95 % confidence interval.
5) Y(1) and Y(2) are Y-intercept for the first and second compartments, respectively.
6) Nos. 101 and 117 are identical with Nos. 201 and 217 in Table 2, respectively.
7) When less than 4 samples were obtained during the first 2 months after end of exposure, $T_{\frac{1}{2}}(1)$ was assumed to be 30 d.
8) Sample obtained 94 d after end of exposure.

Table 2. Kinetics of decrease of blood-lead levels (B-Pb) during a temporary stop of occupational exposure in 17 lead workers observed for less than one year and in 2 volonteers who had a short heavy exposure.

Subject No.[1]	Age[2]	Exposure time	First B-Pb	Observation time	No. of samples	One compartment[6]	Two-compartment model			
							Fast compartment		Slow compartment[6]	
						R^2[3]	$T_{\frac{1}{2}}(1)$ (CI)[4]	Y(1)[5]	Y(2)[5]	R^2[3]
	(y)	(y)	(μmol/l)	(d)		(%)	(d)	(μmol/l)	(μmol/l)	(%)
201[7]	43	3	6.7	189	20	87	26(21-35)	3.6	2.6	97
202	59	23	4.5	209	12	87	43(25-147)	1.2	2.7	92
203[8]	59	22	4.2	172	10	94	69(38-337)	2.1	1.9	96
	60	23	5.4	118	14	96	49(36-75)	2.8	2.3	98
204	53	22	4.0	239	12	88	37(25-74)	1.3	2.2	95
205	38	14	3.9	48	7	89	14(7-oo)	1.2	2.2	92
206	60	5.5	3.0	112	11	92	28(18-61)	1.1	1.5	96
207	52	29	3.0	218	12	73	63(20-oo)	0.5	2.0	75
208[8]	28	3.5	3.4	114	10	94	34(24-55)	1.5	1.7	98
	29	4.0	3.2	115	11	94	24(18-36)	1.2	1.6	99
209	48	7.5	3.5	171	11	78	13(8-38)	1.3	1.8	88
210	58	3.5	3.9	160	11	92	47(28-139)	1.5	1.8	95
211	59	13	2.8	155	12	54	8(5-18)	0.6	1.9	89
212	49	10	3.5	238	13	59	7(5-10)	0.8	2.4	96
213	51	1.5	3.0[9]	120	10	87	20(14-34)	1.9	1.6	97
214	40	1.0	3.5	155	12	97	50(38-75)	2.0	1.0	99
215	50	1.0	3.3	147	9	84	24(19-35)	1.4	1.7	98
216	29	0.3	3.5	83	11	97	42(24-171)	2.4	0.9	97
217[7]	59	26	3.0	111	9	96	63(31-oo)	1.5	1.2	97
318	33	0.0001	2.1	215	12	94	27(17-62)	1.4	0.1	94
319	37	0.0001	2.3	500	13	97	44(32-71)	1.7	0.1	97

1) No. 201 was a cast bronze founder, Nos. 202-205 demolition workers, Nos. 206-217 smelter workers, and 318-319 volunteers.
2) At end of exposure.
3) R^2 is degree of explanation.
4) $T_{\frac{1}{2}}(1)$ is half-time for the fast compartment. CI=95 % confidence interval.
5) Y(1) and Y(2) are Y-intercepts for first and second compartments, respectively.
6) $T_{\frac{1}{2}}$ (2) is assumed to be 5 y.
7) Nos. 201 and 217 are identical with Nos. 101 and 117 in Table 1, respectively.
8) Two subjects were studied twice.
9) Sample obtained 15 d after end of exposure.

The accuracy was tested twice each year in a Nordic inter-laboratory calibration program with 6-19 (mean 12) accepted laboratories participating at each occasion. The regression function of our results (Y, µmol/l) on the average result of the other laboratories (X) was Y=1.008X-0.052. Our results in the 115 samples (range 0.2-5.6 µmol/l) averaged 96.3 per cent (range 80-112 per cent, 62 per cent within 95-105 per cent) of the mean of the other laboratories. Further, we participated in the U.K. External Quality Assessment Scheme during the later part of the study. In the 29 samples analysed, our results by single analysis average 99 per cent (range 90-115 per cent, 72 per cent within 95-105 per cent) of the mean (2.40 µmol/l) of the about 90 participating laboratories. Neither quality control series displayed any time trend.

During the first year of observation in subjects Nos. 104-115, the first three years in subject No. 102, and the six first years in subject No. 116, B-Pb was determined by a colorimetric method after extraction with dithizone in chloroform. The detection limit was about 0.05 µmol/l. The results obtained by the colorimetric method averaged 105 % (SD=6 %) in the concentration range 0.3 - 1.9 µmol/l and 100 % (SD = 5 %); in the range 2.0 - 5.4 µmol/l of results obtained with the flame AAS method.

In subject No. 116, for the following four years, determinations were made by flame AAS after precipitation of proteins with trichloroacetic acid (22). The detection limit was 0.2 µmol/l, and the method error about 10 per cent.

Mathematical analysis.

Three models corresponding to the sum of one, two and three exponentials were considered for the B-Pb decay curves of each individual worker. A fixed "background" value of 0.3 µmol/l was used for all subjects and each model. The non-linear regression procedure in the statistical package BMDP (21) was used. this program produces estimates of the parameters which minimize the unweighted residual sum of squares using a modified Gauss-Newton algorithm. Minimum and maximum values can be specified for each parameter. Thus two parameters and their asymptotic standard deviation were estimated: an elimation rate (transformed and quoted as a half life, $T_{\frac{1}{2}}$ (1), $T_{\frac{1}{2}}$ (2), and $T_{\frac{1}{2}}$(3)) and the concentration corresponding to each compartment (Y (1), Y (2), and Y (3)). Confidence intervals were estimated assuming asyptotic normality of the estimates. The fit of the three models was judged by comparing the fraction of total variance in the B-Pb values explained (R^2 %). In addition, plots of residuals vs. time were used for checks of the validity of the model and the accuracy of the individual curve fittings.

To describe accumulation, a function of the type Y(t)=A(1-exp(-B x t)) was fitted to the data by use of the non-linear regression procedure in BMDP (21). A is a scale constant, B an elimination constant, and t time.

Bone-lead levels (bone-Pb)

Levels in the left middle fore-finger phalanx were determined in vivo by an X-ray fluorescence (XRF) method, as described earlier (15) in 37 subjects. The detection limit was 20 µg/g and the method error about 15 per cent. Readings below the detection limit were assigned a value of 10 µg/g in the calculations. In most cases, levels derived either at duplicate measurements (15) or calculated from a series of measurements (16) were employed.

Statistics

In general, non-parametric tests have been employed. For associations were used the Spearman's rank correlation (r_S) and for comparisons of duplicate measurements in the same individual the Wilcoxon's matched-pairs ranked-sign test. Comparisons between groups were made by the Mann-Whitney U-test. In a few instances, single or multiple linear regression analysis was made. For establishing of inter-individual variations, binomial tests were employed. When more than one observation series was available in a particular individual, the value corresponding to the calculations with the best fit (R^2) of the compartment analysis was used. All p-values are two-tailed. "Statistically significant" denotes $p<0.05$.

RESULTS

The decline rate of B-Pb was, in most cases, rapid soon after end of exposure, later on slower (figure 1). There was generally a good fit of the observed B-Pb to any of the three compartment models tested (tables 1 and 2). However, one subject (No. 122; table 1) displayed bad fits to any of the models, probably because the first sample was obtained 94 d after end of exposure. He will thus be disregarded in the following. Another ex-lead worker (No. 114) was excluded because of suspected lead exposure during the first month of the supposed exposure-free period. Before the second observation period (from year 7 on) his B-Pb had decreased very close to the back-ground level and no conclusions as regards kinetics of the second compartment could be made.

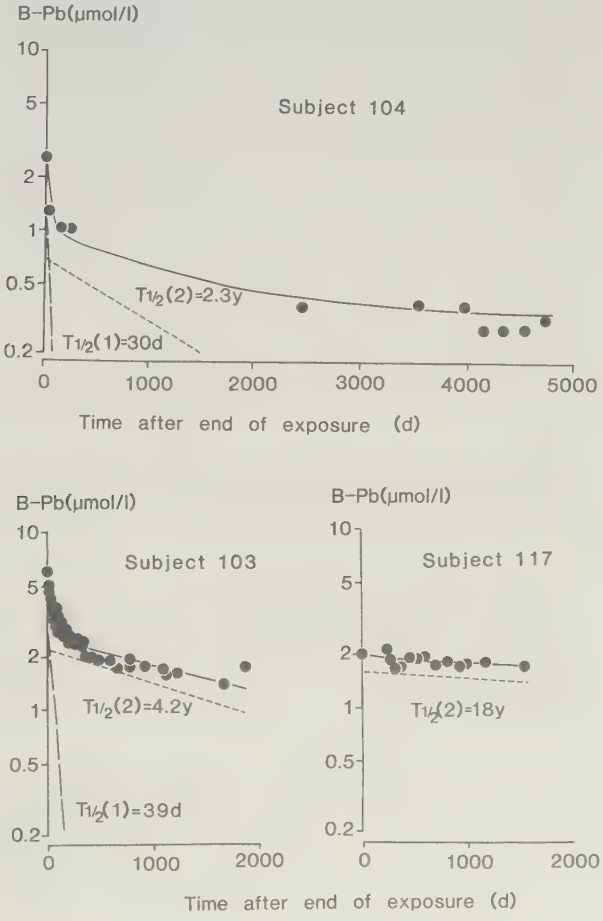


Figure 1. Decline of blood lead level (B-Pb, logarithmic) after end of exposure in ex-lead workers. A two-compartment model is fitted to the data. Half-times ($T_{\frac{1}{2}}$) for the compartments are indicated. In subjects Nos. 104 and 117, the half-time of the fast compartment ($T_{\frac{1}{2}}(1)$) was assumed to be 30 d.

In most ex-lead workers, the number of observations during the first period after end of exposure were too few (less than four per 2 months) to allow reasonably accurate estimates of the decay rate of the fast compartment. Thus, in all but three (Nos. 102, 103, and 123), an approximate half-life of the fast compartment ($T_{\frac{1}{2}}(1)$) of 30 d (see below) was employed.

The fit of the two-compartment model (median 97 per cent, range 35-99) was considerably and significantly ($p<0.0001$, Wilcoxon) better than of the one-compartment model (median 86 per cent; tables 1 and 2). The fit of the three-compartment model was similar (medians 97 vs. 97 per cent; table 1), though significantly ($p<0.01$, Wilcoxon) better.

When employing the three-compartment model, the median of Y(1) was 0.6 (range 0.0 - 2.3) µmol/l, of Y(2) 1.4 (range 0.0-2.5) µmol/l, of $T_{\frac{1}{2}}(2)$ 3.7 (range 0.3 -16) y, of Y (3) 0.3 (range 0.0-2.6) µmol/l, and of $T_{\frac{1}{2}}(3)$ >100 (range 4.9 - oo) y.

In the following, only the simplest model with a good fit, i.e. the two-compartment one, will be discussed.

Using the two-compartment model, the decay rate in the remaining 21 ex-lead workers (table 1) had a median biological half-time of the slow compartment ($T_{\frac{1}{2}}(2)$) of 5.6 y, during a median post exposure period of 13 y. There was a considerable range of $T_{\frac{1}{2}}(2)$, 2.3-27 y (table 1).

The data was sufficient for an estimate of the half-time of the fast compartment in three of the ex-lead workers (Nos. 102, 103, and 123, table 1) and in the 17 temporarily removed lead workers (table 2). The observation period in the latter group was too short for an accurate estimation of half-life of the slow compartment ($T_{\frac{1}{2}}(2)$). Thus, in the calculation of $T_{\frac{1}{2}}(1)$ in these subjects, an approximate $T_{\frac{1}{2}}(2)$ of 5 y (see above) was used, as being the best estimate. The median $T_{\frac{1}{2}}(1)$ in the 20 subjects was 29 d. Their median observation time was 155 d. The range of $T_{\frac{1}{2}}(1)$ was considerable, 7-63 d (table 2; in subjects No. 203 and 208, the $T_{\frac{1}{2}}(1)$ with the best fit, 49 d and 24 d respectively, was used). The decay curves with the shortest and longest $T_{\frac{1}{2}}(1)$, respectively, are shown in figure 2.

When, in two subjects (Nos. 203 and 208; table 2), the decay pattern was studied during two periods of temporary removal from exposure, 0.5 and 1.0 y apart, the decline rates of B-Pb were compatible.

The two formerly occupationally non-exposed subjects had $T_{\frac{1}{2}}(1)$ of 27 and 44 d (table 2).

The Y-intercept of the fast compartment (Y(1)) had a median of 0.8 (range 0.0-3.0) µmol/l in the 21 ex-lead workers (table 1), and 1.3 (range 0.5 - 3.6) µmol/l in the 17 temporarily removed ones (table 2). The difference was statistically significant ($p=0.007$, Mann-Whitney).

The Y-intercept of the slow compartment (Y(2)) had a median of 1.8 (range 0.7-2.6) µmol/l in the 21 ex-lead workers (table 1), and 1.8 (range 0.9-2.7) µmol/l in the 17 temporarily removed workers (table 2). The groups were, of course, not significantly different. In both of the two occupationally non-exposed subjects, Y(2) was 0.1 µmol/l.

In the 21 ex-lead workers, Y(2) made up for a median of 68 (range 33-100) per cent of the combined compartments (Y(1) plus Y(2)), in the temporarily removed workers 57 (range 27-80) per cent. The difference was statistically significant

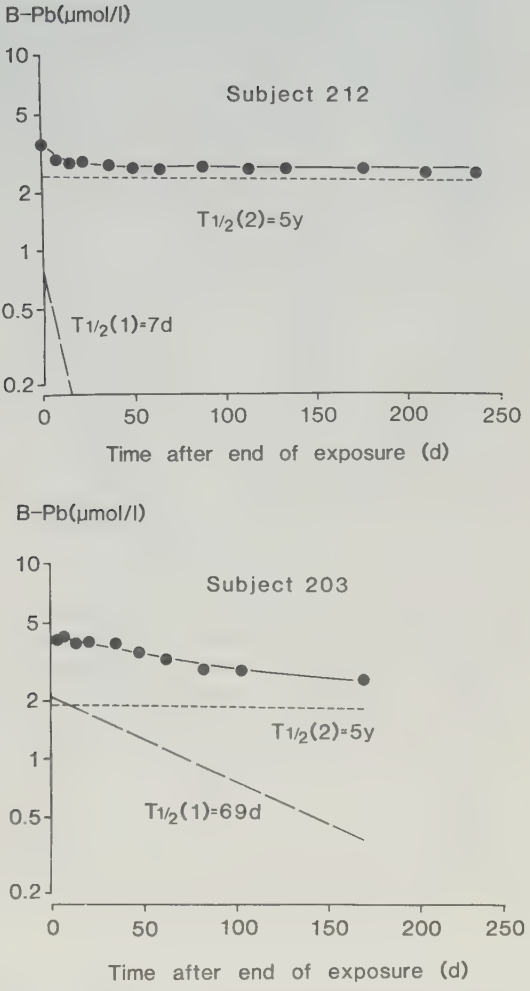


Figure 2. Decline of blood lead level (B-Pb, logarithmic) in two lead workers, temporarily removed from exposure. A two-compartment model is fitted to the data. Half-time ($T\frac{1}{2}$) for the slow compartment was set at 5 y. $T\frac{1}{2}$ for the fast compartment is indicated.

(p=0.02, Mann-Whitney). In all the lead workers, the median was 65 per cent, while the fractions were 6 and 7 per cent in the two occupationally non-exposed subjects.

$T\frac{1}{2}(1)$ correlated significantly with Y(1) (figure 3; r_S=0.41, p=0.03), but not with either age, time of exposure, initial B-Pb, Y(2), or serum creatinine levels. In five out of the 20 subjects with individually estimated $T\frac{1}{2}(1)$, the linear regression line for $T\frac{1}{2}(1)$ upon Y(1) ($T\frac{1}{2}(1)$ = 5.4 x Y(1) + 23), did not run through the 95 per cent confidence interval of $T\frac{1}{2}(1)$. This shows that there is statistically significant (p=0.0004, binomial test) inter-individual variation of $T\frac{1}{2}(1)$.

$T\frac{1}{2}(2)$ in the ex-lead workers correlated significantly with age (figure 4; r_S=0.50, p=0.01), but not with either exposure time, observation time, initial B-Pb, Y(1), Y(2), or serum creatinine levels. In 10 out of the 21 subjects, the linear regression line for $T\frac{1}{2}(2)$ upon age ($T\frac{1}{2}(2)$=0.15 x age - 1.7), did not run through the confidence interval of $T\frac{1}{2}(2)$. This shows that there is an inter-individual variation of $T\frac{1}{2}(2)$ (p<0.0001).

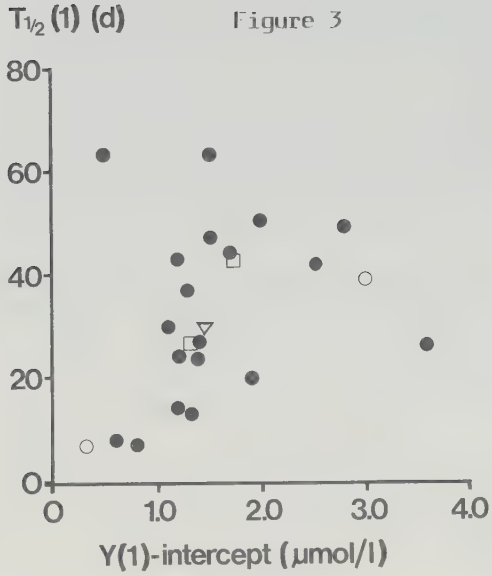


Figure 3

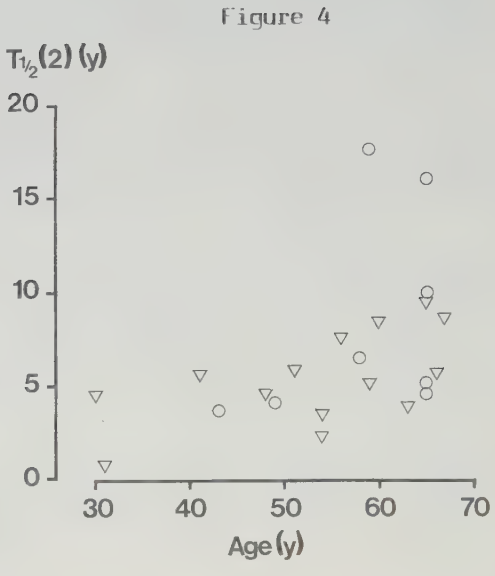


Figure 4

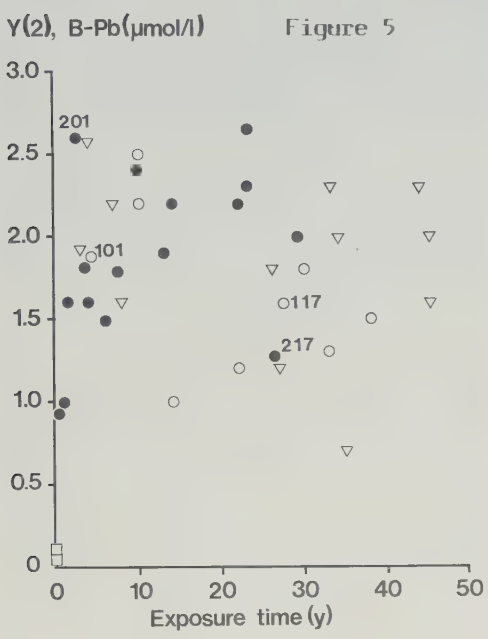
Figure 3. Relationship between half-time ($T_{\frac{1}{2}}(1)$) and the Y-intercept (Y (1)) of the fast compartment in a two-compartment model fitted to the decline of blood-lead levels (B-Pb) after end of exposure. Closed symbols denote lead workers in table 2, open ones ex-lead workers in table 1 (circles: subjects studied for 5 y or less; triangles: subjects studied more than 5 y) and formerly occupationally non-exposed subjects (squares).

Figure 4. Relationship between age and the half-time of the slow compartment ($T_{\frac{1}{2}}(2)$) in a two-compartment model fitted to the decline of blood-lead levels after end of exposure. Symbols as in Figure 3.

When Y(2), in all 38 lead workers and the two non-exposed subjects, was plotted against exposure time (figure 5), there was no significant non-parametric association. Neither was there any linear correlation. However, when an exponential accumulation curve was fitted to the data, there was a reasonable fit (R^2 = 49 %, $p<0.001$). The elimination constant was 1.2, corresponding to a half-time of 0.6 y, and Y(2) levelled off at 1.8 µmol/l.

Moreover, there was a tendency for the temporarily removed workers to have a higher Y(2) at a particular exposure time, than the ex-lead workers, and especially those in the latter group, who were followed for a short time. However, that difference was not statistically significant at multiple regression analysis. Neither did observation time display any significant association with Y(2).

In 35 lead workers and the two non-exposed subjects, there was a significant correlation between Y(2) and bone-Pb and Y(2) (figure 6; r_S=0.36, p=0.01). Multiple linear regression analysis displayed that there was an increase of Y(2) by 0.008 µmol/l per µg/g of bone-Pb (p=0.005). Further, the temporarily removed workers had in average 1.0 µmol/l higher Y(2) than the ex-lead workers. This was also obvious from a decrease of Y(2) by 0.09 µmol/l per year of the post exposure observation time (p<0.0001). In the active workers, there seems to be a levelling off of Y (2) when bone-Pb increased. There was no such clear corresponding tendency in retired workers.



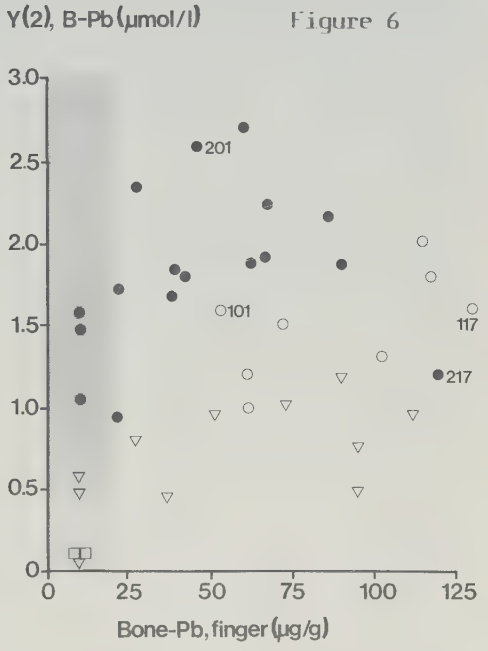


Figure. 5. Relationship between time of occupational lead exposure and Y-intercept of the slow compartment (Y(2)) in a two-compartment model fitted to the decline of blood-lead levels (B-Pb) after end of exposure. Symbols as in figure 3. Two subjects (Nos. 101/201 and 117/217) belong to both groups.

Figure 6. Relationship between lead level in finger-bone (Bone-Pb) and Y-intercept of the slow compartment (Y(2)) in a two-compartment model fitted to the decline of blood-lead levels (B-Pb) after end of exposure. Symbols as in figure 4. Two subjects (Nos. 101/201 and 117/217) belong to both groups. Note: The "Y(2)" values are not the same as in tables 1 and 2, as they have been recalculated to the time of the first bone-Pb determination.

Discussion

A multiple exponential model fitted well to the decay pattern of B-Pb. However, there are other possibilities, e.g. power functions. Indeed, data indicating a non-linear relation between air lead level and B-Pb (e.g. 12, 13, 19), between B-Pb and plasma lead (20, 42, 45) and between B-Pb and the lead level in urine (12, 13, 56, 67), could favour a choice of a non-linear model. However, elimination rates similar to ours have been reported earlier, both in far less (11, 13, 53) and in far more (14, 46) exposed subjects. Also, there was no indication in the present data that elimination rates were faster in subjects with high initial B-Pb; $T_{\frac{1}{2}}(1)$ increased rather with increasing Y(1). Thus, there are at present no serious objections against using a multiple exponential model, at least not in the B-Pb concentration range that we have studied.

In a few subjects, the fit was less good (in five subjects was R^2 <80 %), in one of them bad (R^2=0%). This may be due to the fact that, in those subjects, the B-Pb was low during a major part of the observation period and that thus the analytical error had a great impact.

Is the relevant number of compartments two? Different authors have proposed one (23, 68), two (2, 7, 63, 64), three (4, 33, 43, 53, 66), four (13, 23, 44, 45, 61), and even five (6) compartments in human metabolic models. The simplest model that gave a good fit in the present study was the two-compartment one. Thus, there was no reason to choose a more complicated model. Of course, from a theoretical point of view, a larger number of compartments is probable. Thus, data in the two subjects exposed only to a single heavy lead dose, may indicate an inital very fast decay of B-Pb (56). In most of the subjects in the present study, such a phenomenon would remain undetected: There was probably a continous absorption of lead from the lungs and gastro-intestinal tract for some time after end of exposure (see below). Also, the early observations were few. However, in the two subjects (Nos. 208 and 216) in whom frequent observations were made during the first few days after end of exposure, the data did not indicate any rapid initial decrease. Moreover, in addition to the relatively small number of observations, the limited observation period, and the analytical method error may obscure other compartments, especially small or very slow ones. Indeed, some observations in the present study may indicate that the slow compartment really has more than one component (see below).

Which parts of the body constitute the two compartments? The only organ containing lead amounts sufficiently large to cover the considerable excretion associated with the decay of the slow compartment (in the order of 0.05 - 0.1 mg/24 h in urine only, for several years; 61), is the skeleton, which contains hundreds of milligrams (2, 15, 16, 39, 59). The identity between the slow compartment and the skeletal pool is also strongly supported by the association between Y(2) and bone-Pb. The soft tissues, including the lung, contain only a few mg (10, 59), an amount which fits with the excretion during the emptying of the fast compartment.

The median half-time of the fast compartment was about one month. What errors may effect that estimate? It is difficult to be absolutely sure that the exposure did stop totally at a fixed date; some exposure may have proceeded after the formal end of exposure. Thus, for the temporarily removed smelter-workers, the work-site after removal was located only a few hundred meters from the plant. Also, the homes of the workers may be contaminated. In addition, the worker may have a pool of lead in the lungs and the gastro-intestinal tract and thus continue to absorb lead for some time after stop of lead inhalation and ingestion. The limited data on hand - frequent measurement in two subjects - may indicate such an absorption, but mainly up to one week efter

end of exposure, which is in accordance with earlier observations (9, 12, 13, 31, 32). This delayed absorption is probably the reason of the present weak positive correlation between $T_{\frac{1}{2}}(1)$ and Y(1). These possible sources of error all tend to give a somewhat too long estimate as compared to the true half-life. Further, the temporarily removed workers were not randomly selected, they were removed from exposure because of high B-Pb, which might partly be an effect of a slow elimination rate in those particular individuals. Another possible explanation of the slight positive association between $T_{\frac{1}{2}}(1)$ and Y (1) is a bias introduced by disregarding possible intermediate compartments.

B-Pb is mainly present in the red cells. It could thus be suspected that the life-time of these cells would determine the $T_{\frac{1}{2}}(1)$. However, the calculated $T_{\frac{1}{2}}(1)$ is considerably shorter than would be expected if lead was eliminated from blood only at the normal death of these cells. But lead is known to cause hemolysis. Could that have affected the $T_{\frac{1}{2}}(1)$? Hardly, at least not considerably, as there was no correlation between $T_{\frac{1}{2}}(1)$ and the initial B-Pb; a negative correlation would be expected if hemolysis was important. In the temporarily removed lead workers, we had to employ an estimated $T_{\frac{1}{2}}(2)$ of 5 y. However, this did not affect the $T_{\frac{1}{2}}(1)$; even a $T_{\frac{1}{2}}(2)$ as short as 1 y, or as long as 10 y, would cause only slight changes of $T_{\frac{1}{2}}(1)$.

Having taken these possible errors into consideration, we still find it fully justified to conclude that the average $T_{\frac{1}{2}}(1)$ is about one month. This is also compatible with various kinds of earlier data on elimination (2, 11-14, 20, 27-29, 36, 37, 40, 46, 49, 53, 54, 56, 65), if a second, slow compartment is taken into consideration, and on accumulation (5, 24, 27, 28, 37, 38, 48, 65, 67) of B-Pb.

There was a considerable range in $T_{\frac{1}{2}}(1)$ between different subjects. To some degree this may be explained by various errors in the estimates of individual decay curves. Thus, the two subjects studied twice had different $T_{\frac{1}{2}}(1)$ at the first and second occasion, which could not reasonably be accounted for by real variation. It should be noted though, that the half-times were not statistically significantly different, as the confidence intervals overlapped. It is difficult to explain all the variation within the population by errors; there is most probably a true inter-individual variation in turnover rate, as has been observed for lead in dogs (23), as well as for other heavy metals in man (methylmercury:58; cadmium: 35, 70; chromium: 71), though the true range of $T_{\frac{1}{2}}(1)$ may perhaps not be as large as ten times. The inter-individual variation may be due to differences in excretion, in urine and/or in faeces. However, it was not associated with the serum creatinine levels and there was no association between elimination rate and urinary lead excretion (61). One possibility is a variation in fecal excretion due to different elimination through bile and/or intestinal reabsorption. In fact, there are indications of a considerable inter-individual difference in gastrointestinal absorption (8, 31).

The median half-time of the slow compartment was about five years. Some of the estimates are probably not too accurate, because at lower B-Pbs, the analytical method error is more important. Also, the true "back-ground" B-Pb may vary somewhat between individuals, even in a population as the Swedish, which has low and rather homogenous B-Pbs. Thus, a somewhat lower "back-ground" is indicated in subject No. 104 in the decay curve in fig 1. Further, the number of observations was relatively low and the observation period should preferably have been a couple of decades. In most of the ex-lead workers, we had to employ an estimated $T_{\frac{1}{2}}(1)$ of 30 d. This may have affected the $T_{\frac{1}{2}}(2)$, but only slightly. However, we consider the median of about five years as reasonably correct. Further, there was definitely an inter-individual variation of $T_{\frac{1}{2}}(2)$. But the true range between individuals cannot be considered firmly established.

There was an increase of $T_{\frac{1}{2}}(2)$ with rising age. This does not necessarily mean a decrease of the turnover rate of skeletal lead, but may have other explanations: First, senile osteoporosis may add significantly to the mobilization of lead from the skeleton, thus causing a gradual increase of B-Pb with time, which then leads to a spuriously high $T_{\frac{1}{2}}(2)$. However, there was no association age/$T_{\frac{1}{2}}(2)$, which is consistent with the lack of acceleration of the decrease of bone-Pb with time in subjects followed by repeated XRF measurements of bone-Pb (16, 60). Second, as lead is excreted by glomerular filtration (69), the decrease of this parameter with rising age, may cause a gradual relative increase of B-Pb, which would result in spuriously high $T_{\frac{1}{2}}(2)$. We did not see any association between the serum creatinine level and $T_{\frac{1}{2}}(2)$, but glomerular filtration rate may decrease considerably without any rise of serum creatinine. Another possible explanation of the slight association between $T_{\frac{1}{2}}(2)$ and age is that older subjects were exposed to higher lead levels in the past than at present, and consequently have a larger pool of "old lead" in a slow pool, from which lead is now returning to the blood. However, against the importance of this talks that there was no significant association between time of exposure and $T_{\frac{1}{2}}(2)$.

The presence of a lead pool in the body for a long time after end of exposure has been obvious from a series of investigations (e.g. 15, 16, 26, 29, 47, 52). The main lead pool is in the skeleton (1, 2, 15, 16, 39, 57, 59). Much has been obscure considering the kinetics of the skeletal lead pool. The $T_{\frac{1}{2}}(2)$ (e.i. elimination from the skeleton) of five years reported here is faster than most earlier estimates of the half-life of skeletal lead (8-71 y) by different techniques (6, 18, 23, 30, 34, 51, 62, 63, 66). However, it is in accordance with data from some other studies (3, 17, 25, 68, 72, 73). Also, it fits remarkably well with results of repeated measurements by XRF of finger-bone-Pb in ex-lead workers (a few of whom are identical with the present subjects; 16, 60). There are several possible explanations of the differences: The method employed, the part of the skeleton assayed (in our case a reflex of the lead release from the whole skeleton), and the observation time in relation to exposure.

The Y-intercepts, Y(1) and Y(2), reflect the influence on B-Pb by the fast and the slow compartment, respectively. The relation between Y(1) and Y(2) varied considerably in different individuals, probably because of the exposure history. Thus, in an individual with a short heavy exposure, the Y(1) should dominate, while in subjects exposed for many years, though only slightly lately, Y(2) should be more important. It is interesting to note that over-all in the present study, Y(2) made up for as much as 1.8 µmol/l, corresponding to 65 per cent of B-Pb, and that it ranged as far as up to 2.7 µmol/l and 100 per cent. This is a larger fraction than has earlier been assumed (2, 12, 30, 41, 53). The difference may be due to the intensity and time of exposure and the time of observation. The large impact of skeletal lead on B-Pb is important from a practical point of view, as the fraction of B-Pb which mirrors the slow compartment, decreases only slowly, even after complete removal from exposure.

As mentioned above, the slow compartment probably mainly reflects the lead level in the skeleton, which is strongly supported by the association between Y(2) and finger-bone-Pb. The retired workers were lower in Y(2) in relation to finger-bone-Pb than the active ones. In the former, Y(2) may mainly reflect the lead pool in the slow bone-Pb compartment (53, 63), represented by the mainly cortical (or compact) finger-bone, in the latter the fast trabecular (or cancellous) bone pool may be more important (57). But, in addition, in the active workers, Y(2) may also be affected by a small, intermediately fast pool, perhaps contained mainly in the liver, the kidneys (10, 59), and the skeleton (an even faster pool than the trabecular one). When closely looking at the data of subject

No. 103 in figure 1, one may, in fact, anticipate more than one component in the slow compartment.

The impact on Y(2) by other organs than the skeleton may be indicated by the accumulation pattern of Y(2) with rising exposure time. A steady state was reached already within a couple of years. This is faster than in trabecular bone (57); the turnover of cortical bone is much slower (16, 60). The shape of the accumulation curve may also be affected by the non-linear behaviour of lead in erythrocytes (19, 40, 43). This may also be the cause of the apparent levelling of Y(2) upon finger-bone-Pb in active workers (but not in the retired ones, who had lower B-Pbs).

There was a considerable inter-individual variation in Y(2) at a certain exposure time. One obvious explanation is variations in intensity of exposure. An additional explanation may be the inter-individual variations in lead metabolism seen in this study, as well as earlier ones (8, 31, 61).

The variation in kinetics of lead metabolism should mean a considerably varying risk for different individuals exposed at the same level, which, of course, is important from a practical point of view. A short $T_{\frac{1}{2}}$ (1), as the result of a rapid excretion, is probably an advantage for the worker. On the contrary, a long $T_{\frac{1}{2}}$ (2) may be good, as this means that the net endogenous exposure from the skeleton is low.

In many countries, lead workers are removed from lead exposure when they reach a B-Pb "trigger level" ("removal level"; in Sweden at present 3.0 µmol/l), and are not allowed to return until B-Pb has decreased to a "safe level" ("return level"; 2.0 µmol/l). In our "typical" lead worker, who has an Y(2) of 1.8 µmol/l, and a $T_{\frac{1}{2}}$(1) of one month, this will take as much as about 6 months. However, newly employed workers, who have a small Y(2), would display the same decay in less than a month. On the other hand, as many as 57 per cent (24/42) of our workers had an Y(2) of more than 1.7 µmol/l. Assuming a "back-ground" level of 0.3 µmol, they would reach 2.0 µmol/l only after sufficiently long time has elapsed to affect the slow compartment. In the workers who were followed for a long time, 30 percent (7/23) would require more than a year to reach the "safe level". These assumptions are in accordance with observations in removed workers (50).

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Lead in Finger-Bone Analysed In Vivo in Active and Retired Lead Workers

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In 75 active lead workers the median lead level in finger-bone (bone-Pb), as determined in vivo by an X-ray fluorescence method, was 43 μg/g (range $<$20–122). In 32 retired workers the median level was even higher, 59 μg/g (range $<$20–135), which indicates a slow turnover rate of lead in finger-bone. This was confirmed in 18 of the "active" workers, in whom bone-Pb was studied in connection with an exposure-free period. In spite of a significant decrease in blood-lead levels (B-Pb), no systematic change of bone-Pb occurred. There was an increase of bone-Pb with time of employment, but with a large interindividual variation. No association was found between bone-Pb and present B-Pb in the active lead workers. However, in the retired ones, B-Pb rose with increasing bone-Pb. The bone-lead pool thus causes an "internal" lead exposure.

Key words: lead, bone, skeleton, in vivo, X-ray fluorescence, occupational, retirement, metabolism, toxicokinetics, blood

INTRODUCTION

In lead-exposed subjects the lead accumulates mainly in bone. The skeleton contains over 90% of the total body burden [Task Group on Metal Accumulation, 1973]. The available information on levels and kinetics of lead in bone is limited.

There are several studies on skeletal lead levels (bone-Pb) in autopsy material from subjects not occupationally exposed to lead, by chemical analysis [Flemming, 1964; Schroeder and Tipton, 1968; Grandjean, 1973; Grandjean and Holma, 1973; Barry, 1975; Gross et al, 1975; Ulrich, 1978] or by X-ray fluorescence analysis (XRF) [Wielopolski et al, 1983]. However, only limited data have been published on bone-Pb in lead workers, and then mainly in dead ones [Flemming, 1964; Grandjean and Holma, 1973; Barry, 1975; Lindh et al, 1978, 1980]. In addition, a small number of analyses of bone-Pb in living-lead exposed subjects have been reported, based upon chemical analysis of biopsy material [Westerman et al, 1965] or in vivo XRF

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[Ahlgren et al, 1976, 1980a; Eastwell et al, 1983]. However, there is a definite need for more information.

It is generally assumed that the turnover rate of bone lead in man is slow [Task Group on Metal Accumulation, 1973]. However, quantitative estimates of the kinetics are uncertain, based either upon extrapolation from animal data [Black et al, 1968], upon calculations by various metabolic models [Holtzman, 1960; Jaworowski, 1967; Holtzman et al, 1968; Rabinowitz et al, 1976; Smith and Hursh, 1977; Ahlgren et al, 1980a], upon incorporation of tracer lead in the skeleton [Rabinowitz et al, 1976], or upon indirect conclusions from the decrease rate of blood-lead levels (B-Pb) after end of exposure [Skerfving et al, 1983].

Here we report in vivo determinations of bone-Pb in active and retired lead workers as well as repeated measurements in connection with an exposure-free period.

SUBJECTS STUDIED AND METHODS

Subjects

Active lead workers. Seventy-five males were studied. The average age was 46 years (SD 11, range 19–65). They had a mean exposure time of 10 years (SD 12, range 0.1–37). All but two had been exposed within two weeks before measurements; in two workers the last exposure had occurred six and eight months before examination. Fifty-six subjects were smelter workers, six were brass founders, four had been scrapping red-leaded goods, seven were storage-battery plant workers (of whom one had also worked in a plastic factory using lead stearate as a stabilizer for polyvinyl chloride), one was a spray-painter who had used red lead oxide paint, and one had produced such pigment.

Retired lead workers. Thirty-two male former smelter workers who had not been exposed for the last year or longer (mainly due to retirement) were studied. The average age was 68 years (SD 5.3, range 53–80). The mean exposure time was 24 years (SD 9.8, range 4–43). The average time after end of exposure was 4.3 years (SD 2.8, range 1–13).

Examinations

An occupational and medical history, including alcohol habits, was obtained from each individual. A determination of bone-Pb was made. Moreover, in 16 smelter workers and two scrappers who were removed from exposure, mainly due to high B-Pb (usually about 3.5 μmol/liter or more), a new measurement of bone-Pb was made after an exposure-free period of 2–11 months. Venous blood samples were analyzed for B-Pb, hemoglobin level, sedimentation rate, and red and white cell counts, as well as calcium, phosphate, and creatinine concentrations, and alkaline phosphatase (S-ALP) and gamma-glutamyl transferase (S-GT) activities in serum. A urine sample was analyzed for albumin and glucose.

Methods

In vivo determination of bone-Pb by XRF. The measurements have been carried out in vivo on the second phalanx of the left forefinger, according to an XRF method [Ahlgren et al, 1976, 1980a,b, 1981; Ahlgren and Mattsson, 1979]. The forefinger was fixed in a polymethylmethacrylate holder and about 1 cm^3 of the

second phalanx was irradiated for 40 minutes with two collimated ^{57}Co sources (86% 122 keV, 10% 136 keV). The collimation of these sources was oppositely directed and the total activity was 0.8 GBq. The count rate of the characteristic X-rays of lead was measured with a high purity Ge-detector (16 mm [diameter] × 5.2 mm [thick]; energy resolution: Full Width Half Maximum [FWHM] = 750 eV at 75 keV). The detector was connected via a charge-sensitive preamplifier and a pulse-shaping linear amplifier to an 80 MHz analog-to-digital converter. A baseline restorer was used to maintain the energy resolution at the high count rates used. The mean angle between the incident and measured radiation was 90°. The mean diameter of the examined finger-bone was calculated from two orthogonal radiographs.

Bone-Pb was then calculated by comparing the in vivo measurements with measurements on cylindrical fingerlike phantoms consisting of an inner core simulating the bone and an outer part simulating the surrounding soft tisues [Ahlgren and Mattsson, 1979]. The inner core was made of silica paraffin wax and bone ash with known amounts of lead added to it. The outer part consisted only of silica paraffin wax.

The accuracy of the method was also verified by studies of a phalanx which had been measured in vivo (1.2 years before death; Table I) in a smelter worker. The naked middle phalanx-bone was analysed by nondestructive XRF using same geometry as was employed in the in vivo measurements. By using the quotient of the lead K_α X-rays and the incoherently scattered radiation, the lead concentration could be estimated [Ahlgren et al, 1981]. Thus the calibration was different from the in vivo procedure, in which calibration by a phantom was employed. Then the bone was analysed chemically by flame atomic absorption spectrometry (AAS) after combined wet and dry digestion and extraction with dithizone in methyl isobutyl ketone.

The minimum detectable concentration of bone-Pb for the in vivo measurements was 20 μg/g (signal level corresponding to three times the square root of the number of background counts). The precision as studied by double measurements in 16 persons having bone-Pb in the range 27–122 μg/g (mean 70; Fig. 4) was 10 μg/g (SD of differences, 15% of the mean).

The absorbed dose to the central part of the irradiated finger during one measurement is 2.5 mGy [Ahlgren et al, 1980a].

B-Pb determination. Venous blood was obtained in heparinized acid-washed tubes. B-Pb determinations in connection with bone-Pb measurements were made by a method described earlier [Schütz and Skerfving, 1976]. The sample was wet-ashed. Lead was extracted with dithizone in methyl isobutyl ketone and determined by AAS. Repeated interlaboratory checks have shown good accuracy. The limit of detection

TABLE I. Determinations of Lead in Finger-Bone (μg/g) in a Lead Worker In Vivo and Post Mortem by X-Ray Fluorescence (XRF) and Atomic Absorption Spectrometry (AAS)

		Post mortem	
Section	In vivo XRF	XRF	AAS
Proximal	—	129	129
Middle	98	95	91
Distal	—	136	127

was 0.05 μmol/liter (1 μmol/liter = 21 μg/100 ml) and the precision 4%. Double determinations were always made.

Time integrated B-Pb. In the smelter and the storage-battery plant B-Pb had been analyzed at least four times yearly in each worker for up to 11 years before the bone-Pb study. Three different methods of B-Pb analysis were employed at different time periods. Up to 1–3 years before bone-Pb determinations, a spark emission spectroscopy method was used after dry ashing. Later, two different AAS methods were employed, both including wet digestion and extraction (in the last period the method described above). The full period of occupational lead exposure was covered in 43 of the workers in whom bone-Pb was determined (39 smelter workers and 4 storage-battery workers). Their average age was 41 years (SD 11, range 19–65) and their mean time of exposure 5.1 years (SD 3.3, range 0.1–11). B-Pb was plotted as a function of time (Fig. 1). The mean B-Pb at time of bone-Pb determination was 2.4 μmol/liter (SD 1.3, range 0.77–3.5). As the primary interest was in the occupational exposure, a level of 0.5 μmol/liter, which is the approximate "background" B-Pb level found in unexposed Swedes [Elinder et al, 1983], was subtracted from each B-Pb determination. Then a time-integrated B-Pb was estimated by a graphical method and expressed as μmol × year/liter.

Statistics. In general nonparametric tests have been employed. For comparison of groups the Mann-Whitney U-test has been used; for comparison of repeated measurements in the same individual, the Wilcoxon matched-pairs ranked-sign test. Correlations have been calculated by Spearman's rank method (r_S). In two instances linear multiple regression analysis has been made. All p values are two-tailed.

RESULTS

The median bone-Pb in the 75 active lead workers was 43 μg/g (range <20–122; Fig. 2). Their average B-Pb at the time of bone-Pb determination was 2.6 μmol/

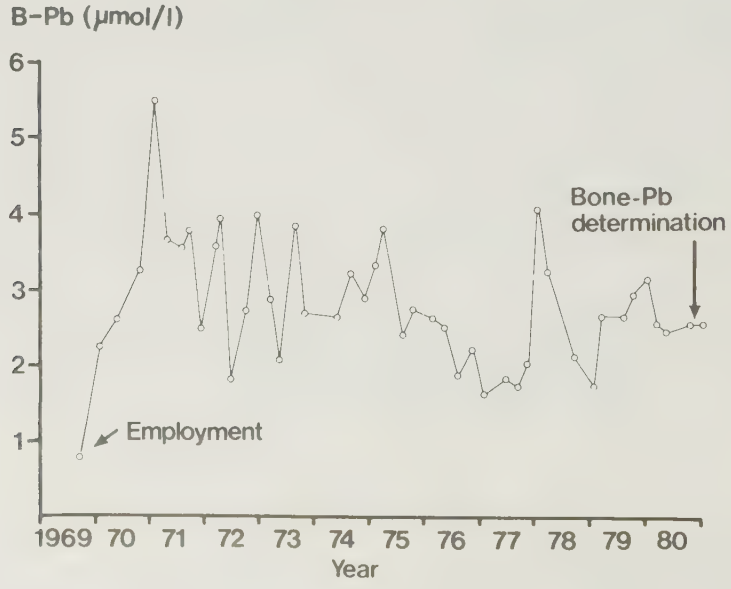

Fig. 1. Blood-lead levels (B-Pb) in a smelter worker. Dips in B-Pb were generally caused by removal from lead exposure (at >3.5 μmol/liter).

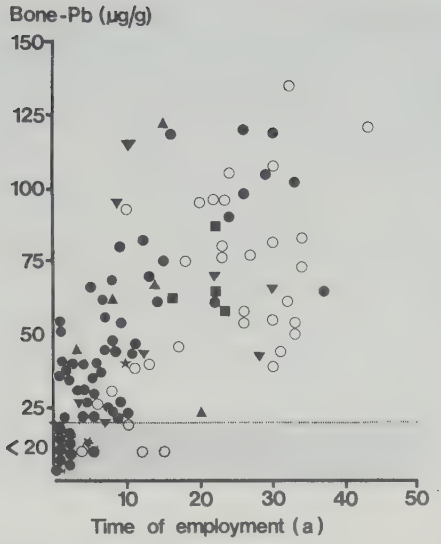

Fig. 2. Bone-lead levels (bone-Pb) and time of employment in 75 active (closed symbols) and 32 retired (open symbols) lead workers. Circles denote smelter workers; squares, scrappers; triangles pointing downwards, storage-battery workers; triangles pointing upwards, metal moulders; stars, a painter and a worker in a paint-pigment production plant. The area below detection limit is shadowed.

liter (SD 0.75, range 0.77–5.0). The median bone-Pb in the 32 retired lead workers was 59 µg/g (range <20–135, Fig. 2). Their average B-Pb was 1.2 µmol/liter (SD 0.34, range 0.39–1.8). The difference in bone-Pb between active and retired lead workers was statistically significant (Mann-Whitney test, $p = .04$), as was B-Pb ($p < .00001$).

Bone-Pb rose with time of employment (Fig. 2; $r_S = .49$, $p = .002$). However, the variation of bone-Pb with time of employment was considerable. In a few workers bone-Pb was well above the detection limit already within a year of exposure. After ten years of employment the maximum level obtained was about 100 µg/g.

As bone-Pb may be affected not only by time of employment but also by age, category (active/retired), and time between end of exposure and bone-Pb determination, further analysis was made by multiple regression. It revealed an average linear increase of bone-Pb with about 2 µg/g per year of employment ($p < .001$), and in addition, a significant difference of bone-Pb between retired and active (on average 24 µg/g higher than in retired ones, $p = .01$) workers, but no significant effects on bone-Pb of either time after end of exposure (average increase 3 µg/g per year, $p = .1$) or age (decrease 0.05 µg/g per year, $p = .9$).

The mean time-integrated B-Pb during time of occupational exposure in 43 workers was 9.6 µmol × year/liter (SD 7.2, range 0.05–28). Bone-Pb was correlated with integrated B-Pb (Fig. 3; $r_S = .41$, $p = .003$). However, again the variation was considerable. Linear regression analysis displayed an average linear increase of about 2µg/g per µmol × year/liter ($p < .0001$). To determine the effect of each of the two dimensions of the time-integrated B-Pb, ie, time of employment and average B-Pb, they were evaluated separately by multiple regression analysis. There was a significant impact of exposure time (3 µg/g per year, $p = .001$), but not of mean B-Pb (4 µg/g per µmol/liter, $p = 0.4$).

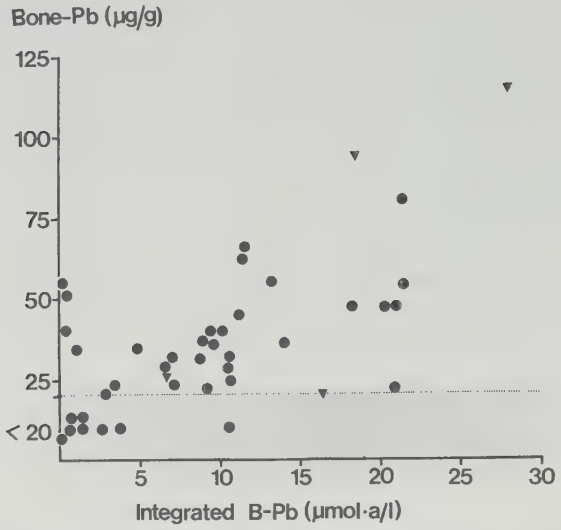

Fig. 3. Bone-lead levels (bone-Pb) and time-integrated blood-lead levels (B-Pb) in 43 lead workers. Symbols as in Figure 2.

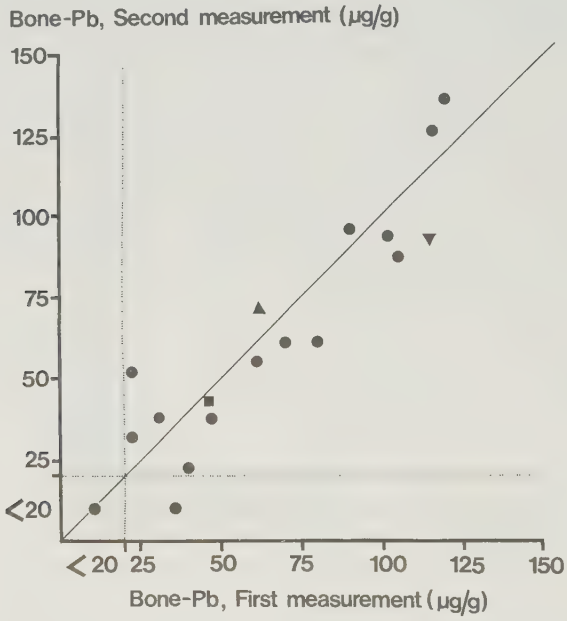

Fig. 4. Bone-lead determinations (bone-Pb) in 18 lead workers before and after an exposure-free period averaging five months. A line of identity is shown. Symbols as in Figure 2.

In 18 workers bone-Pb determination was repeated after 2–11 months (average 5.3). Between the measurements they were not occupationally exposed to lead. There was no significant change of bone-Pb (Fig. 4; Wilcoxon's test, $T = 58$, $p = .2$). In the 16 subjects, who had both readings above the detection limit, the mean of the second measurements was 105% (range 56–236%) of the first one. The initial mean B-Pb was 2.9 µmol/liter (SD 0.82, range 0.39–5.0). The B-Pb decreased in all workers, to an average of 71% (range 51–90%) of the initial value, which is

statistically significant ($T = 0$, $p < .01$). There was no association between the B-Pb decrease rate and bone-Pb in individual workers.

In active lead workers there was no statistically significant association between bone-Pb and B-Pb (Fig. 5; $r_S = .05$, $p = .2$). However, a significant association was present in retired subjects ($r_S = 0.42$, $p = .01$), although there was a great interindividual variation. On average, a bone-Pb of 100 μg/g was associated with a B-Pb of about 1.5 μmol/liter.

One active lead worker was clinically slightly lead poisoned (upper abdominal pain, constipation, neuropathy, and slight anemia) at the time of the bone-Pb study. He had the seventh highest bone-Pb (115 μg/g). Five subjects had earlier been treated in hospital for lead poisoning. All had bone-Pb above the mean. The blood sample showed slight anemia in three persons and slightly increased S-ALP and S-GT in nine (two of whom were alcoholics). One subject had a slight increase of creatinine and another a slight decrease of calcium level in serum. None of these biochemical analyses displayed any clear-cut association with bone-Pb measurements.

DISCUSSION

The concentrations of lead in finger-bone of the lead workers were generally high, sometimes over 100 μg/g, which is far higher than the levels reported in bone of unexposed Scandinavians [a few μg/g; Grandjean, 1973; Grandjean and Holma, 1973; Lindh et al, 1980]. The present observations are in agreement with findings by the same method in a few former workers in a smelter [Ahlgren et al, 1976] and a storage-battery plant [Ahlgren et al, 1980a]. The levels encountered are generally higher than those recently recorded with a similar method in petrol sniffers [Eastwell et al, 1983].

There are variations in the lead content in different parts of the skeleton, especially between cortical and trabecular bone, levels being higher in the former

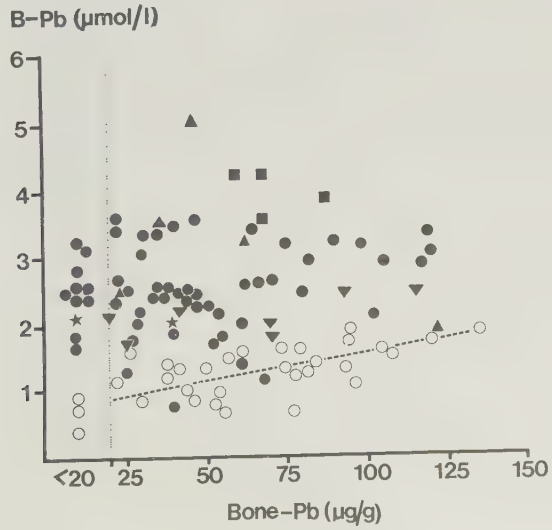

Fig. 5. Lead levels in bone (bone-Pb) and blood (B-Pb) in 75 active and 32 retired lead workers. Symbols as in Figure 2. The linear regression line ($Y = 0.0059X + 0.90$) denotes open symbols above the detection limit.

[Barry, 1975; Gross et al, 1975; Ulrich, 1978]. The finger-phalanx bone is mainly of cortical type, at least in the middle part of the phalanx. Only limited information is available on levels in finger-bone in relation to other parts of the skeleton. The data on hand indicate that the concentration is lower than in femur but similar to vertebra [Skerfving et al, 1983]. Taking these facts into consideration, our data may be compared with information published earlier on levels in other parts of the skeleton. The present levels thus are much higher than those found with other methods in subjects not exposed to lead occupationally [Flemming, 1964; Westerman et al, 1965; Schroeder and Tipton, 1968; Grandjean, 1973; Grandjean and Holma, 1973; Barry, 1975; Gross et al, 1975; Ulrich, 1978]. Also, the concentrations are higher than most of the ones reported on occupationally exposed persons [Flemming, 1964; Grandjean and Holma, 1973; Lindh et al, 1980]. In a few studies of a limited number of lead workers, levels comparable to the present ones were found [Barry, 1975; Lindh et al, 1978]; the highest concentrations reported were about two times higher than ours [Westerman et al, 1965].

The lead in finger-bone is metabolized slowly. This is clearly shown by the lack of decrease in the subjects studied twice at beginning and end of a period free of occupational exposure. Although the time of observation was certainly rather short, the results exclude the presence of a substantial fast lead pool in the finger-bone. The slowness of bone-lead metabolism is also shown by the fact that as long as several years after end of exposure retired lead workers still had bone-Pb significantly higher than those found in still active ones.

The retired workers had a substantially longer average time of exposure than the still active ones. When the influence of the exposure time on bone-Pb had been eliminated using the multiple regression analysis, the retired subjects had significantly lower average bone-Pb than the active ones. There are several possible explanations for this difference. It may be due to the fact that in the regression analysis was used a simple linear model, which is presumably not fully adequate. Thus the impact of the long exposure time in the retired workers was probably overestimated. Moreover, although we could not prove a decrease of bone-Pb with time after retirement, such a change might still have occurred. Also, aging of the skeleton may contribute.

There was a general increase of bone-Pb with time of employment, but also a considerable variation of bone-Pb at the same time of employment. Lead contamination of the skin, still persisting after the rigorous cleaning procedure before measurement, may have caused some of this variation by false high readings. However, as no systematic decrease occurred in workers studied a second time after an exposure-free period, contamination should not be important. There are other explanations. Thus the intensity of the exposure has varied considerably between different industries. Also, as conditions of occupational hygiene have improved continuously, the exposure has decreased with time in the same plant. We tried to eliminate some of these effects by employing B-Pb, which is usually considered to mirror absorption, in a time-integrated dose-index. However, the variation was still large. One possible reason for this is an interindividual variation of the rate of incorporation and/or mobilization of skeletal lead. This is in agreement with the variation in half-time of B-Pb earlier encountered [Skerfving et al, 1983].

The accumulation pattern of bone-Pb with time of exposure may indicate the presence of different lead pools. Thus, in some subjects the initial increase was

surprisingly rapid, within a year the levels were above the detection limit of our method, and thus higher than in unexposed subjects [Ahlgren et al, 1976, 1980a]. This may be due to loading of one pool. But, in addition, some of it may reflect skin contamination. Later on, the accumulation rate seemed to be slower, a top level of about 100 μg/g was recorded after ten years. This increase rate is probably rather extreme and reflects an unusually high exposure, which is indicated by the fact that many of the workers studied had been temporarily removed from exposure because of high B-Pb.

Our data might indicate a bone-Pb plateau after a couple of decades of exposure. This is compatible with a pool having a half-time of about half a decade, which is also indicated by other observations [Skerfving et al, 1983], but shorter than has usually been assumed [Holtzman, 1960; Jaworowski, 1967; Holtzman et al, 1968; Task Group on Metal Accumulation, 1973; Smith and Hursh, 1977].

In some studies of occupationally unexposed subjects, bone-Pb rose with age [Barry, 1975; Gross et al, 1975]. We found no such clear-cut impact. This may be due to the fact that the "background" exposure in Swedes is low [Schütz, 1979; Elinder et al, 1983; Schütz et al, 1984] in comparison with the occupational exposure. A small effect of age could easily have been masked.

The bone-Pb may be useful, on an individual or group basis, as a time-integrated index of lead absorption in cross-sectional studies of, for example, dose-response relationships. The connection with time-integrated B-Pb supports this. The large variation may seem disappointing. However, the time-integrated B-Pb which we compared to bone-Pb is an oversimplification. Since lead deposited in bone long ago may have been removed due to the turnover of the skeleton, the impact of a certain B-Pb on the present bone-Pb should be time-dependent. To solve the B-Pb/bone-Pb relationship, a metabolic compartment model must be employed. Such a model should make it possible to transform a certain bone-Pb level into an approximate curve of B-Pb over time, which would be valuable epidemiologically.

B-Pb is dependent upon absorption and excretion of lead as well as upon incorporation and mobilization of lead pools of the body [Grandjean and Kon, 1981]. In active lead workers, B-Pb is, of course, profoundly affected by recent exposure. It is thus not surprising that these workers displayed no association between B-Pb and bone-Pb. However, in retired workers there was such a correlation. In the latter group the external exposure is less important, and thus, B-Pb is more dependent upon lead mobilized from the skeleton. This may be important as retired workers therefore suffer an "internal" lead exposure. At a bone-Pb of 100 μg/g, B-Pb was on average about 1 μmol/liter above the "background" level in Swedes [Elinder et al, 1983]. Further, if the outflow of lead from the skeleton is not realized, elevated B-Pb, which is really due to "historical" exposure, may be misinterpreted as caused by present lead exposure. This dilemma can be solved by determinations of bone-Pb in vivo.

It has been claimed that a large skeletal lead pool may constitute a serious risk if it is rapidly mobilized because of pathological conditions in the bone [Grandjean, 1983]. However, although theoretically possible [Ahlgren et al, 1980a], there are no convincing case reports on such events [Waldron and Stöfen, 1974].

Determinations of bone-Pb by XRF should thus be a useful method both in biological monitoring of lead workers and in research. The precision is acceptable; the figure of 15% reported here is, of course, a maximum, as it was calculated from duplicate measurements separated by an exposure-free period, which means that some

real changes—though no systematic change was demonstrated—cannot be excluded. A drawback of the present setup is the relatively high detection limit, which is well above the "normal" levels. However, work is in progress to lower it.

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Decrease of skeletal lead levels in man after end of occupational exposure

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ABSTRACT

Lead levels in finger-bone were monitored using an in vivo X-ray fluorescence technique in retired lead workers. Eight subjects followed for 2-5 yr directly after end of exposure all displayed a decrease. Their average half-time was 7 (range 3-15) yr. In a second group of six persons, followed from year 7 to year 13 after finishing lead work, a decrease was seen in all but one. The average half-time for this group was 8 (range 2-oo) yr. The mean value for both groups was 7 yr. The results show that there is a decrease of lead in bone after end of exposure and that it is considerably faster than earlier estimated from various data on lead metabolism.

INTRODUCTION

Lead accumulates in the skeleton, which may contain more than 90 per cent of the body burden.[1] The knowledge on the kinetics of this large pool is scanty. The turnover rate is generally assumed to be very slow.[2] However, the evidence for this is circumstantial, based mainly upon animal studies,[3] upon the rate of bone remodeling,[4] upon various lead balance models,[5-11] or upon indirect observations of the decrease rate of blood-lead levels (B-Pb) after end of exposure.[1,12]

Direct measurements on skeletal lead kinetics in man are almost lacking. We have earlier reported that repeated determinations in lead workers by in vivo X-ray fluorescence analysis (XRF) during 5 months after end of exposure did not indicate the presence of a substantial fast pool in finger-bone.[13] But conclusions on the turnover rate of the slow pool(s) require longer observation periods.

As skeletal lead affects B-Pb,[13] as bone lead (bone-Pb) has a potential use as a time-integrated dose-index,[13] and as a large bone-Pb pool may constitute a potential health hazard if mobilized rapidly,[10, 14-15] firm data on the kinetics are needed.

We here report data on repeated in vivo measurements bone-Pb after end of occupational exposure.

MATERIALS AND METHODS

Subjects studied. In 1979-82, eight persons (group 1) were included in the study. They consisted of seven smelter workers and one storage battery worker. Their average age at the time of the first measurement was 61 (range 49-64) yr, and the exposure time 24 (10-38) yr (Table 1). Measurements were made at least once a year. The time between end of exposure and first measurement was 1-359 (median 38) d. The number of bone-Pb measurements ranged 4-9 (median 7). The follow-up time was 1.6-4.5 (median 4.2) yr.

In a second group, consisting of six former storage battery plant workers (group 2), bone-Pb was first determined in 1978, 7 yr after end of exposure.[10] New determinations were made in the period 1982-84. Their average age at the time of the first measurement was 56 (37-72) yr, and their exposure time 26 (3-45) yr (Table 1). The number of bone-Pb measurements was 5. The total bone-Pb follow-up time was 6.0 yr.

Table 1. Results from the mono-exponential fit to the follow-up of lead levels in finger-bone, according to the expression Bone-Pb(t) = A · e^{kt}. At the first measurement t=0. T($\frac{1}{2}$) denotes the estimated biological half-time. In addition, half-time of the slow compartment in a two-compartment analysis of blood-lead levels (B-Pb) is shown.

Group	Subject No.	Age (yr)[1]	Exposure time (yr)	Bone-Pb A ($\mu g \cdot g^{-1}$)	Bone-Pb k (yr^{-1})	Bone-Pb T($\frac{1}{2}$) (yr)	Bone-Pb Coefficient of correlation	B-Pb $T_{\frac{1}{2}}$ (yr)
1	1-1	59	26	141	-0.205	3.4	-0.78	18
	1-2	65	38	72	-0.132	5.2	-0.71	16
	1-3	61	24	79	-0.121	5.7	-0.64	
	1-4	58	22	53	-0.120	5.8	-0.26	6.5
	1-5	65	14	47	-0.102	6.8	-0.29	4.6
	1-6	65	26	130	-0.0544	13	-0.48	5.1
	1-7	49	10	114	-0.0492	14	-0.57	4.2
	1-8	65	33	104	-0.0471	15	-0.37	9.5
	Mean	61	24	93	-0.104	6.7[2]	--	7.8[2]
2	2-1	37	3	95	-0.283	2.4	-0.85	4.6
	2-2	61	35	95	-0.126	5.5	-0.72	3.5
	2-3	63	26	52	-0.0789	8.8	-0.23	7.6
	2-4	48	10	27	-0.0126	55	-0.05	5.6
	2-5	58	34	73	-0.0111	62	-0.07	5.8
	2-6	72	45	90	0.00455	oo	0.06	9.4
	Mean	56	26	72	-0.0845	8.2[2]	--	5.5[2]
1+2	Mean	59	25	84	-0.0956	7.3[2]	--	6.8

1) At first measurement.
2) Calculated from k.

In vivo measurements of lead in finger-bone (bone-Pb). The measurements have been carried out in vivo on the second phalanx of the left forefinger, according to an XRF technique.[10, 13, 16-18] The Pb atoms in the fingerbone were excited by the γ-radiation from ^{57}Co (122 keV and 136 keV) and the characteristic K_α X-rays emitted from Pb were subsequently detected by a planar, high purity Ge-detector. The experimental set-up is shown in Fig. 1.

Bone-Pb was calculated by comparing the in vivo measurements with measurements on cylindrical finger-like phantoms consisting of an inner core simulating the bone and an outer part simulating the surrounding soft tissues.[17] The inner core was made of silica paraffin wax and bone ash with known amounts of lead added. The outer part consisted of silica paraffin wax only.

In the measurement performed in 1978, a different detector, different collimators, and slightly different calibration procedures were used. The results from these measurements have been recalculated using the same calibration technique as in 1979-84.

A good accuracy of the method has also been ascertained by studies of a phalanx obtained from a dead smelter worker, who earlier had been measured in vivo.[13] The minimum detectable concentration of bone-Pb was 20 μg/g (signal level

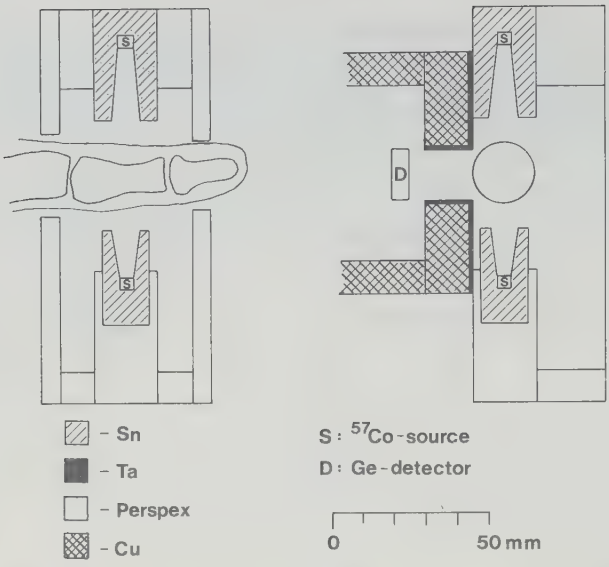

Fig. 1. Arrangement for the determination of lead in finger-bone by X-ray fluorescence analysis. Radiation shield, two ^{57}Co sources, detector collimator with its tantalum-covered opening, and perspex-holder for the finger are shown.

corresponding to three times the square root of the number of background counts). The precision as studied by double measurements in 16 persons having bone-Pb in the range 27-122 µg/g (mean 70) was 10 µg/g (S.D. of differences; 15 % of the mean).

B-Pb determinations. B-Pb was analysed at least at each bone-Pb measurement, generally more often.[1,12] Venous blood was obtained in metal-free heparinized tubes. B-Pb determinations were made by a method described earlier.[19] The sample was wet-ashed. Lead was extracted with dithizone in methyl isobutyl ketone and determined by flame atomic absorption spectroscopy. Repeated inter-laboratory checks have shown a good accuracy. The limit of detection was 0.05 µmol/l and the precision 4 %. Double determinations were always made.

Analysis of bone-Pb and B-Pb as a function of time. A single exponential function of the type $\text{Bone-Pb}(t) = A \cdot e^{k \cdot t}$ was fitted to the bone-Pb determinations using the least square method. In the expression, t denotes time after end of exposure, A level at end of exposure, and the decay rate constant. When a measurement was below the detection limit of 20 µg/g, a value of 10 µg/g was assigned.

For the B-Pb, the sum of two exponential functions and a constant of 0.3 µmol/l was employed[1,12] The constant corresponds to the "background" average B-Pb in persons in Scania without occupational exposure. The slow compartment is assumed to indicate bone-Pb. As the information during the first period after end of exposure was too scanty to permit an accurate, individual estimate of the kinetics of the fast compartment, a half-time of 30 d was employed, which is the median of a larger material.[1,12].

Other examinations. An occupational and medical history was obtained from each individual. All but one subject had, at least once, been removed temporarily from exposure because of high B-Pb (usually 3.5 µmol/l or more) or high urinary delta-aminolevulinic acid levels. Most of them had in that connection suffered from tiredness, gastrointestinal symptoms, and/or joint pains. One worker was slightly lead poisoned (epigastralgias, constipation, neuropathy, and slight anemia) at the time of the first bone-Pb determination. One subject had a slight type 2 diabetes and another one a clinically silent chronic lymphatic leukemia.

Venous blood samples were analysed at least once for hemoglobin level, as well as for calcium, phosphate, and creatinine concentrations, and alkaline phosphatase and gamma-glutamyl transferase (S-GT) activities in serum. S-GT was raised in two subjects, both known to abuse alcohol. All other tests were within the reference limits.

RESULTS

In group 1, followed for 2-5 yr after end of exposure (Fig. 2 A and 2B), the mean initial bone-Pb was 97 (range 61-131) µg/g. All eight showed decreasing bone-Pb with time. The average half-time was 6.7 (range 3.4-15) yr (Table 1). In the same subjects, the half-time of the slow B-Pb compartment had a mean of 7.8 (4.2-) yr. An example of a B-Pb curve is shown in Fig. 3.

In group 2, followed during the period 7-13 yr after end of exposure (Fig. 4), the mean bone-Pb level at first measurement was 72 (range 37-96) µg/g. A decrease was observed in five persons, although the scattering was considerable after the 11th yr. The mean half-time was 8.2 (range 2.4-oo) yr (Table 1). In the same subjects, analysis of B-Pb on time displayed a mean half-time of 5.5 (3.5-9.4) yr for the slow compartment.

With respect to the decay rate constant (k), the two groups are not significantly different (Mann-Whitney U-test, 2p=0.4). Combining the results of the two groups gives a mean of $-0.096\ yr^{-1}$, which corresponds to a half-time of 7.3 yr.

There was no difference in half-times in blood between the two groups (Mann-Whitney U-test, 2p=0.3). When the groups were combined, the average half-time in blood was 6.8 yr.

There were no significant correlations between age and half-time in the skeleton (Spearman's rank correlation, r=0.20, 2p=0.24), between half-time in the skeleton and exposure time (r=0.20, 2p=0.25), or between half-times in the skeleton and blood in individual workers (r=-0.06, 2p=0.41; Table 1). Neither was there any systematic difference between half-times in bone and blood (Wilcoxon matched-pairs signed-ranks test, 2p=0.3).

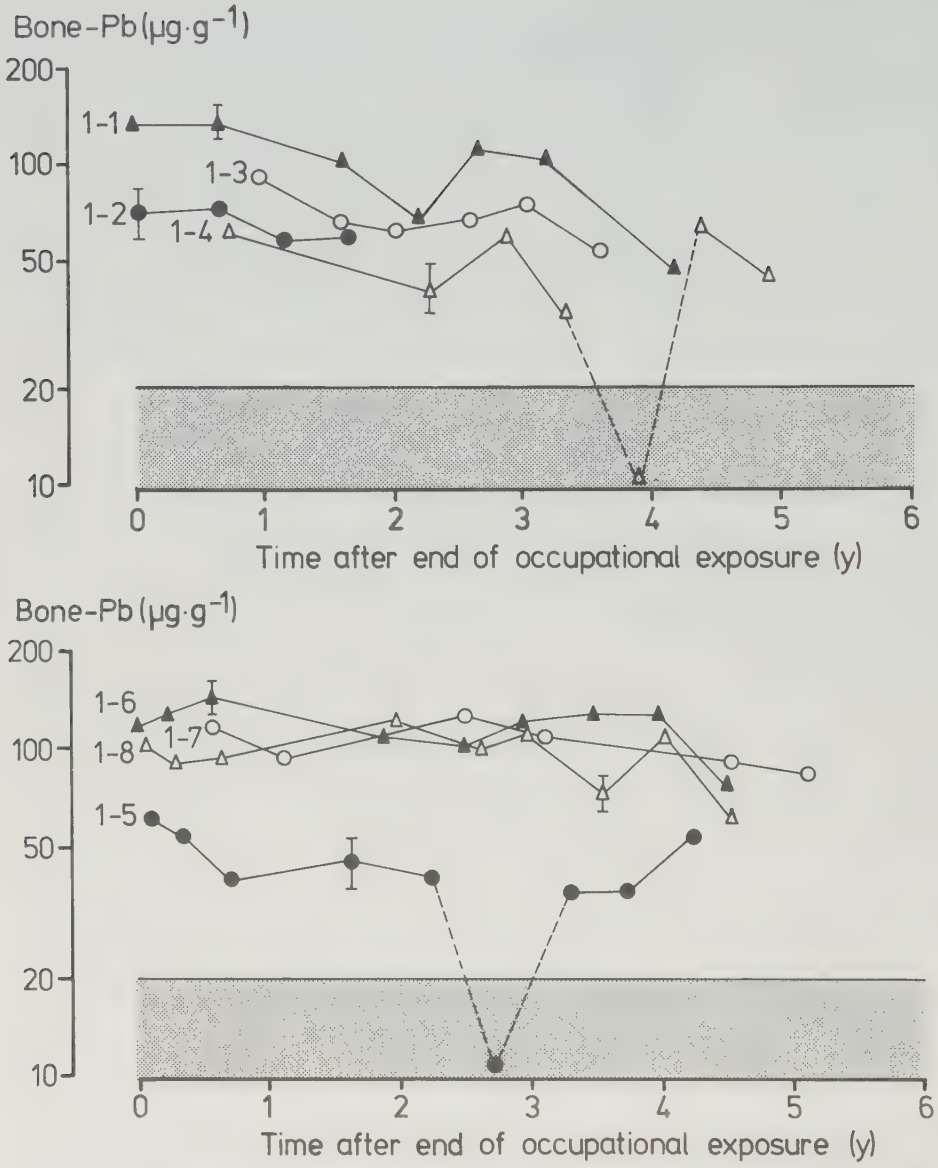

Fig. 2. Finger-bone lead levels (Bone-Pb) on time after end of occupational lead exposure in seven smelter workers and one storage battery worker (Nos. according to Table 1). Area below detection limit is shadowed. The bars indicate the uncertainty (1 S.D.) of the measurements due to counting statistics.

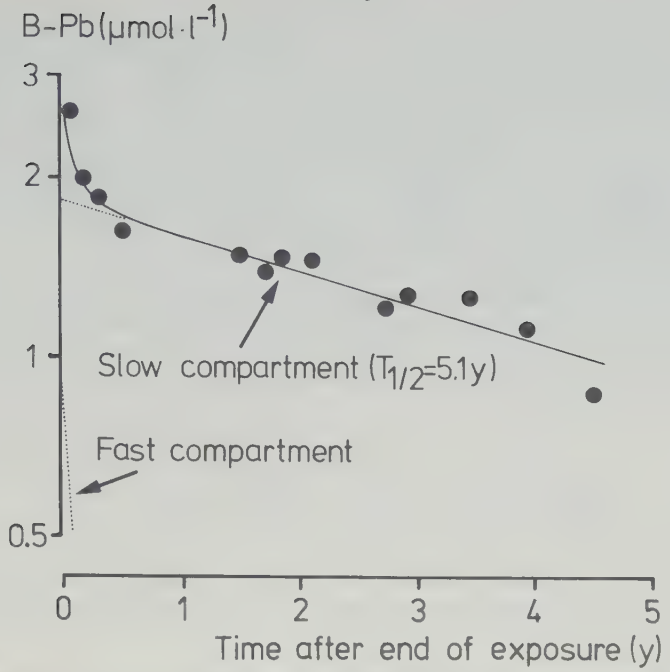

Fig. 3. Blood-lead level (B-Pb) on time after end of occupational lead exposure in a smelter worker (Subject 1-6 in Table 1). A "background" level of 0.3 μmol/l has been subtracted from each B-Pb value. A two-compartment model was fitted to the B-Pb data. Half-time of the fast compartment was assumed to be 30 d. Half-time ($T_{\frac{1}{2}}$) of the slow compartment is given.

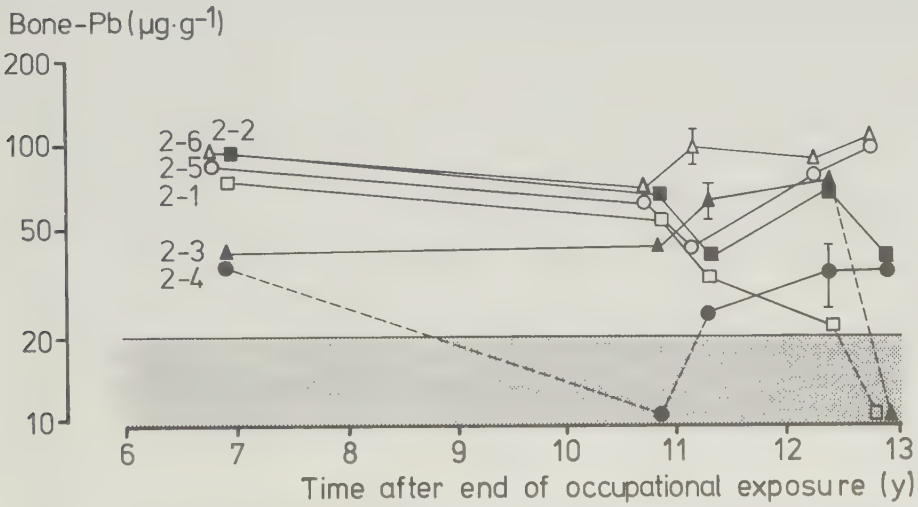

Fig. 4. Finger-bone lead levels (Bone-Pb) on time after end of occupational lead exposure in six former storage battery workers.

DISCUSSION

The present data for the first time directly demonstrates that there is an elimination of lead from bone after end of exposure, but it is slow. The results are in accordance with our earlier reports of no detectable decrease of bone-Pb in workers followed up to half a year after end of exposure[13] and of high bone-Pb in retired lead workers.[10,13,16,20] The levels are, still 13 yr after end of exposure, much higher than in "normal" Scandinavians.[20-23]

Taking into consideration the limitations set by the precision of the method, our data still indicate an inital half-time of about half a decade. This is compatible with conclusions reached from observations of the finger-bone lead accumulation pattern in lead workers[13,24] and B-Pb elimination curves in former lead workers.[1]

Contamination by lead of the skin at first measurements is probably not the reason for the decrease in the present workers, as no decrease was seen in workers followed during half a year after end of exposure.[13]

A systematic error in the first measurement in group 2 cannot be excluded, due to the use of different detectors and collimator, even if the same calibration phantoms were used. However, this error should hardly exceed 10 %, and thus cannot significantly affect the conclusions.

Three of the workers in group 2 deviated from the rest; they had a very slow decrease (one of them even showed increasing bone-Pb). This may have several explanations: The limited number of measurements and their distribution in time causes some restrictions as regards the possibilities to reach firm conclusions on the half-times in individuals. Further, the turnover rate may be age-dependent, e.g. because osteoporotic bone loss. However, these three subjects were not younger than the others, and they even included the oldest individual, 83 yr at the last measurement.

Moreover, the mono-exponential elimination model may not be valid, as there may exist different bone-lead pools. There are several reasons to assume more than one pool: Different parts of the skeleton have varying turnover rates.[25] The turnover rate of lead is more rapid in trabecular than in cortical bone, both in animals[26] and in man.[8,11,20] Finger-bone contains both trabecular and cortical bone. This may be the cause of the different lead levels in ends and shaft reported earlier.[13] Due to the design of the experimental set-up, we believe that, in the in vivo measurements of lead in finger-bone, both types of bone contribute to the signal, even if cortical bone dominates. Thus, a difference between groups 1 and 2 (the latter having longer half-time) might be expected, due to a greater impact of the relatively rapid turnover rate of lead in trabecular bone in group 1, which was studied closer to end of occupational exposure, than in group 2. Indeed, group 2 had a slightly longer mean half-time than group 1.

The half-time for skeletal turnover, as calculated from the ^{90}Sr fractional transfer rate from bone, is 2.4 yr for trabecular bone and 9.5 yr for cortical bone.[25] An assumption that finger-bone consists of 80 % (by weight) cortical and 20 % trabecular bone, and that the concentration of lead at the end of exposure is equal in both types of bone, gives a half-time for the period year 0-5 of 7 yr. Corresponding values for year 7-13 is 9 yr, and for year 0-13, 8 yr. Our measured data concerning bone-Pb were 7, 8, and 7 yr, respectively. However, our

estimates, in addition to mobilization of lead from bone, were affected by recycling of lead into the skeleton. Thus, the turnover of lead may be faster than of ^{90}Sr.

There was a general good accordance between the decay rates in bone and the slow compartment in blood, the mean values of both being 7 yr (all subjects). The lack of correlation in individuals can be explained by the errors in both methods. Also, B-Pb may reflect other bone-Pb pools than those represented in finger-bone. Thus, the elimination from blood (slow component) should reflect the lead turnover rate of the whole skeleton. However, considering the similarity between half-times in finger-bone and blood, finger-bone seems to be a good index of elimination from the whole skeleton.

Our results show in general shorter half-time as compared with calculations from different metabolic models.[4-11]

This work was supported by grants 79-72 and 82-160 from the Swedish Work Environment Fund. Professor Birgitta Haeger-Aronsen, M.D., supplied clinical information on some of the subjects. Assistance was given by Miss Gertrud Lennartsson, Mrs. Ulla Lovén, Miss Marie-Louise Odhnoff, Miss Kristina Parker, Miss Gertrud Persson, Mrs. Ingalill Sandell, and Mr. Nils-Erik Winqvist.

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Lead in vertebral bone biopsies from active and retired lead workers

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ABSTRACT. Samples of vertebra bone were obtained by skeletal biopsy. Lead concentrations were determined by atomic absorption spectroscopy. The median level of lead in bone in 27 active lead workers was 29 µg/g wet weight (range 2 - 155), corresponding to 370 µg/g calcium (range 30-1,120). In nine retired workers the corresponding levels were 19 µg/g (5-76) and 250 µg/g calcium (60-700), and in 14 reference subjects without occupational exposure 1.3 µg/g (1-4) and 13 µg/g calcium (8-40). The bone lead content rose with time of exposure. Comparison of the levels in vertebra with those in finger-bone, as measured by in vivo X-ray fluorescence in the same subjects, strongly suggested the presence of lead pools with different kinetics: The lead turnover rate was more rapid in the mainly trabecular vertebral bone than in the mainly cortical finger-bone. The accumulation pattern, as well as the relation between levels in vertebra and finger, suggest a half-time of lead in vertebra of 1-2 years, which is much faster than usually assumed. Further, there was an association between vertebra and blood lead levels, especially in the retired workers, which shows a considerable endogenous lead exposure from the skeletal pool.

INTRODUCTION

Lead accumulates in the skeleton,[1] which in lead workers may contain more than 90 per cent of the body burden.[2,3] The bone lead burden is not inert, but causes an endogenous lead exposure, which may be considerable.[3-5]

The available information on this large skeletal lead pool is limited, even if determinations by X-ray fluorescence (XRF) has made repeated in vivo analysis of the lead levels in bone (bone-Pb) in lead workers possible, and has supplied useful information.[4-11] However, the relatively high detection limit of the XRF method causes restrictions. Also, the levels,[3] and possibly the turnover rates, of lead vary in different parts of the skeleton. Another possibility to obtain information on skeletal levels in vivo is to make bone biopsies. However, that method has only been used in a limited number of cases.[12-15]

We here report on chemical lead analyses of skeletal biopsies obtained from vertebrae in groups of active and retired lead workers, as well as results of in vivo XRF analysis of lead in finger-bone.

SUBJECTS STUDIED

Active lead workers - Twenty-seven males were studied. Their average age was 46 yr (range 26-65) and their mean exposure time 11 yr (range 0.6-39). All subjects but one had been occupationally exposed to lead within two weeks before biopsy, everybody within seven months. Twenty-one subjects were smelter workers, three were brass founders, one had been scrapping red-leaded goods, one was a storage battery plant worker (who, in addition, had earlier worked in a plastic factory, using lead stearate as a stabilizer for polyvinyl chloride), and one was a spray-painter, who had used red lead oxide paint.

Retired lead workers - Nine male former smelter workers, who had not been exposed (in most cases due to retirement) for an average of 2.6 yr (range 2.3-5.6) were studied. Their mean age was 67 yr (range 61-71) and their mean exposure time 29 yr (range 6-46).

Reference subjects - Two males, who had not been exposed to lead occupationally, were studied. Their age was 36 and 41 yr. In addition, "biopsy" samples were taken from another 12 subjects at autopsy. Their average age was 62 yr (range 34-88). Their causes of death were mainly myocardial infarction or suicide. At least two "biopsies" were analysed from each autopsy case.

Examinations - Determinations of bone-Pb in vertebra (biopsy) and finger (XRF) were made. In the active workers determination of lead in vertebra were performed on average 0.5 yr after measurement in finger (range from 3.5 yr before up to 0.7 yr after). In the retired workers the corresponding mean time was 1.4 yr after (range from 1.6 yr before up to determination at the same time).

Venous blood samples were analysed for blood lead levels.

One active lead worker was clinically slightly lead poisoned (epigastralgias, constipation, neuropathy, and slight anemia) at the time of the bone-Pb study.

METHODS

Determination of lead in vertebra bone - Bone biopsies were obtained from a spinal process of a lumbar vertebra (LIV, LV, or LVI) by use of Radner's instrument.[16] After local anaesthesia of skin and periost, a stainless steel cannula (inner diameter 1.6 mm) was hammered 10-20 mm into the bone, the bone cylinder within the cannula was fractured at its base by rotation of the cannula, and the cannula with its bone content (mean 16.0 mg, range 7.7-23.6) was removed. The sample was immediately placed in an preweighed acid-washed polyethylene tube.

The study was approved by the ethical committee at Lund University. All participants gave written informed consent. No medical complications occurred.

The fresh weight of the samples was determined and they were transferred into acid-washed glass tubes. The dry weight was obtained after three h drying at 120 °C in an oven. Lead and calcium (Ca) contents were determined after the following combined wet and dry ashing procedure: 0.20 ml concentrated nitric acid (Merck Suprapur) was added and the glass tubes were heated for 1.5 h at 120 °C in a heating block. Then the temperature was gradually increased during one h to 280 °C. After cooling, another 0.20 ml aliquot of nitric acid was added and the heating procedure was repeated. Again, after cooling, 0.20 ml of an acid mixture (95% nitric acid, 5% perchloric acid) was added and the samples were heated gradually to 430 °C during two h. The salt residue was dissolved in 0.20 ml concentrated nitric acid and 9.8 ml deionised water. In each series of 24 samples 4-6 blanks were included.

Lead content was analysed by electrothermal atomic absorption spectrometry (AAS), using the method of standard addition. Thus, sample aliquots of 0.50 ml were mixed with 0.60 ml 2% nitric acid including 0.050 µg lead standard in one of the samples. The analytical equipment was Perkin-Elmer AAS 5000 (HGA 500/AS 40/PRS-10). Twenty µl was injected and dried at 100 °C (ramp 15 s; hold 10 s), ashed at 600 °C (ramp 10 s; hold 15 s), and atomized at 2,300 °C (10 s, miniflow 50 ml Ar/min). The absorbance was measured at 283.3 nm. Deuterium background corrector was used.

Calcium was determined by flame AAS after dilution 100 times with 2% nitric acid containing 1% lanthanum.

The detection limit for lead was about 0.01 µg per sample, corresponding to about 1 µg/g wet weight and 10 µg/g Ca. The reproducibility of sampling and analysis was checked by analysing eight biopsies taken with the Radner instrument from two spinal processes of a dead lead worker (Table 1).

Table 1. - Analysis of eight "biopsy" samples from spinal processes of two vertebrae in a dead lead worker.

Parameter		Mean	Range	Coefficient of variation
Fresh weight,	mg	12.7	7.6 - 18.6	29
Dry weight,	mg	8.0	5.3 - 12.2	34
	%	62	52-70	10
Calcium level,	% of fresh weight	9.8	8.2 - 12.0	14
	% of dry weight	15.8	12.6-20.6	16
Lead level,	µg/g fresh weight	73	58-89	16
	µg/g dry weight	119	96-161	18
	µg/g Ca	750	660-780	5

The accuracy of the method was also obtained by analysing 12 biopsies taken at autopsy from a subject without known lead exposure. Into six of the samples, 0.5 µg lead was added before ashing. The average recovery was 105% (S.D. 6.2; range 98-114) after correction had been made for the lead originally present in the six samples without lead addition (mean 3, S.D. 0.9, range 2.0-4.4 µg/g dry weight). Further, analyses of finger-phalanx bone by AAS were in accordance with XRF determinations.[4]

A considerable loss (about 50%) of lead occurred when the ashing procedure was carried out on pure water standards. The loss was avoided by adding 1 mg Ca (as chloride) prior to ashing.

Determination of lead in finger-bone - Measurements were carried out in vivo on the second phalanx of the left forefinger, according to an XRF method.[4,6,7,17]

Bone-Pb was then calculated by comparing the in vivo mesaurements with measurements on cylindrical finger-like phantoms containing silica paraffin wax and bone ash with known amounts of lead added to it. The accuracy of the method was also confirmed when comparing the in vivo XRF results on a smelter worker with the results obtained on the same phalanx post mortem by use of XRF as well as flame AAS.[4] The minimum detectable concentration of bone-Pb

was 20 µg/g (signal level corresponding to three times the square root of background count). The precision as studied by duplicate measurements in 16 persons having bone-Pb in the range 27-122 µg/g (mean 70) was 10 µg/g (S.D. of differences; 15% of the mean).

The finger bone-Pb results have earlier been reported as part of a larger material.[4]

Determination of blood lead (B-PB) - Venous blood was obtained in metal-free tubes (Vacutainer^{R}). B-Pb determinations in connection with bone-Pb measurements were made by a method described earlier.[18] The sample was wet-ashed. Lead was extracted with dithizone in methyl isobutyl ketone and determined by flame AAS. Repeated inter-laboratory checks have shown a good accuracy. The limit of detection was 0.05 µmol/l (1 µmol/l = 21 µg/100 ml) and the precision four per cent. Duplicate determinations were always made.

Time integrated B-Pb - In the smelter and the storage battery plant, B-Pb had been analysed at least four times yearly in each worker for up to 13 years before the bone-Pb study. Three different methods of analysis had been employed at different time periods: Up to 1-3 years before bone-Pb determinations, a spark emission spectroscopy method was used after dry ashing. Later, two different AAS methods were employed, both including wet digestion and extraction (in the last period the method described above). The full period of occupational lead exposure was covered in 16 of the workers (15 smelter workers and the storage battery worker) in whom bone-Pb was determined. Their average age was 41 yr (range 26-62) and their mean time of exposure 5.6 yr (range 0.6-13). B-Pb was plotted *vs.* time. Their mean B-Pb at time of bone-Pb determination was 2.3 µmol/l (range 0.8-7.2). A "background" level of 0.3 µmol/l was subtracted. The B-Pb *vs.* time curves, plotted on paper, were cut out and weighted. Thus a time-integrated B-Pb, expressed as µmol/l x yr, was obtained.[4]

Statistics - In general, non-parametric tests have been employed. For comparison of groups, the Mann-Whitney U-test has been used, for comparison of vertebra and finger measurements in the same individual, the Wilcoxon matched-pairs ranked-sign test. Further, Spearman's rank correlation (r_S) was used. Also, in a few cases, single (r) and multiple linear regression analysis has been made. All p-values are two-tailed. "Significant" denotes $p<0.05$.

RESULTS

The median bone-Pb in vertebra in the 27 active lead workers was 29 µg/g wet weight (range 2-155), and 370 µg/Ca (range 30-1,120; fig. 1). Their median finger bone-Pb was 40 µg/g (range <20-115; fig. 2).

The median vertebra bone-Pb in the nine retired lead workers was 19 µg/g wet weight (range 5-76), and 250 µg/g Ca (range 60-700; fig. 1). Their median finger bone-Pb was 83 µg/g wet weight (range <20-135; fig. 2).

The median calcium concentration in vertebra was in the active workers 102 mg/g wet weight (range 34-129), in the retired ones 84 mg/g (range 43 - 106), and in the controls 106 mg/g (range 67-145). The differences between the groups were not statistically significant, nor was there any correlation between calcium concentration and age ($r = -0.07$; $p = 0.3$).

The vertebra bone-Pb in the active workers was not significantly different from the retired ones, neither as calculated on basis of wet weight ($p=0.4$), nor on basis of calcium content ($p=0.9$), while for finger-bone, the level in retired workers was significantly higher than in the active ones ($p=0.05$).

The median vertebra bone-Pb in the 14 reference subjects was 1.3 µg/g wet weight (range 1-4), and 13 µg/g Ca (range 8-40; fig. 1). Of course, these levels were significantly lower than in both active and retired lead workers ($p<0.0001$). The finger bone-Pb in the two living reference individuals was <20 µg/g wet weight. The B-Pb in the same two subjects was 0.3 µmol/l (both).

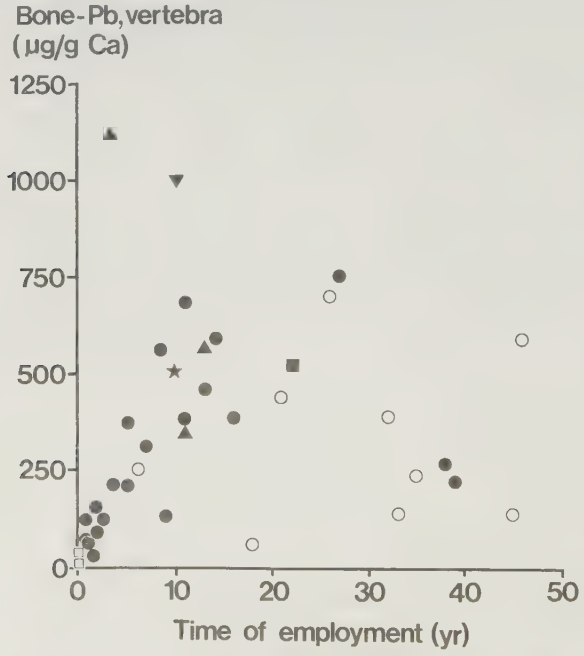

Fig. 1. Lead levels in vertebral bone (Bone-Pb) and time of employment in 27 active (closed symbols) and nine retired (open symbols) lead workers. The circles denote smelter workers, the closed square a scrapper, the triangle pointing downwards a storage battery worker, the triangles pointing upwards metal moulders, and the star a painter. Fourteen referent subjects are indicated by the two open squares.

In the total material of lead workers and the two living reference subjects, there was a correlation between bone-Pb in finger and vertebra, the latter both as expressed on wet weight (r_S=0.62, p=0.001) and on calcium (fig. 2; r_S=0.64, p=0.001) basis.

In active workers with finger bone-Pb above the detection limit, there was no systematic difference between wet weight concentrations in finger and vertebra (p = 0,3, Wilcoxon matched-pairs ranked-signs test). The median ratio was 1.3, (range 0.3-13; n=21). There was no correlation between these ratios and the exposure time (r_S=0.02; p = 0.5). In the retired workers, the levels in finger were systematically higher than in vertebra (median ratio 4.0, range 1.3-6.0; n=8; p=0.01). Accordingly, the ratios between bone-Pb in finger and vertebra in the retired workers were higher than in the active ones (fig. 2; p=0.04, Mann-Whitney U-test).

Vertebra bone-Pb rose with time of employment (fig. 1; r_S=0.77, p=0.001), even if the variation of bone-Pb on time was considerable.

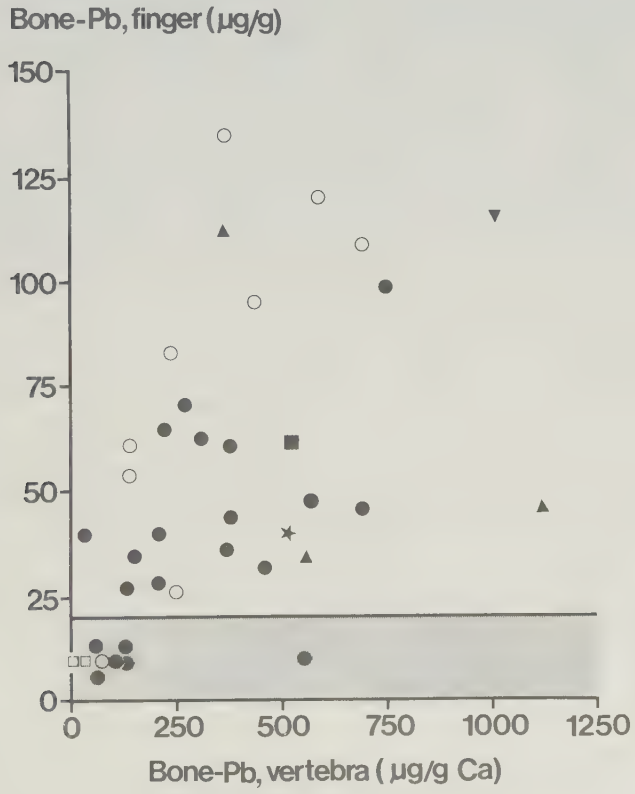

Fig. 2. Lead levels in vertebral and finger-bone in 27 active and nine retired lead workers and in two reference subjects. Symbols as in fig. 2. The area below detection limit for finger-bone-Pb determinations is shadowed.

The median time-integrated B-Pb during time of occupational exposure in 16 active workers was 10.5 µmol/l x yr (range 0.4-31). Vertebra bone-Pb rose on integrated B-Pb, both on wet weight (r_S=0.93, p=0.001) and calcium basis (fig. 3; r_S=0.82, p=0.001). Regression analysis displayed an average linear increase of about 1.8 µg/g wet weight or 22 µg/g Ca per µmol/l x yr.

The median present B-Pb in active workers was 2.3 µmol/l (0.8-7.2), in retired ones 1.4 µmol/l (0.7-1.7). The difference was statistically significant (fig. 4, p=0.0002).

There was a statistically significant association between vertebra bone-Pb and present B-Pb (fig. 4; r_S=0.50, p=0.001). Such an association was seen both in active (r_S=0.44, p=0.01) and retired (r_S=0.93, p=0.001) workers. The ratio between B-Pb and bone-Pb was higher in active than in retired workers (p=0.02, Mann-Whitney U-test). The difference between low and high bone-Pbs corresponded to a difference in B-Pb of about 1 µmol/l in retired workers.

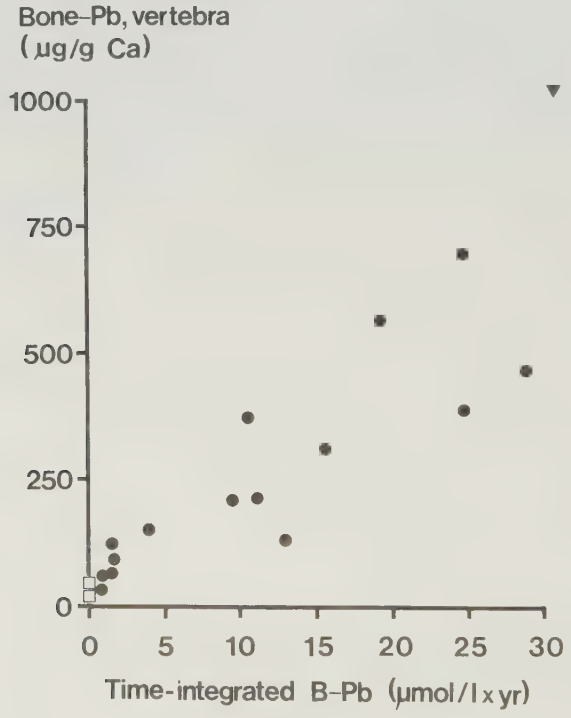

Fig. 3. Lead levels in vertebral bone (Bone-Pb) and time-integrated blood-lead levels (B-Pb) in 16 active lead workers and in two referent subjects. Symbols as in fig. 2.

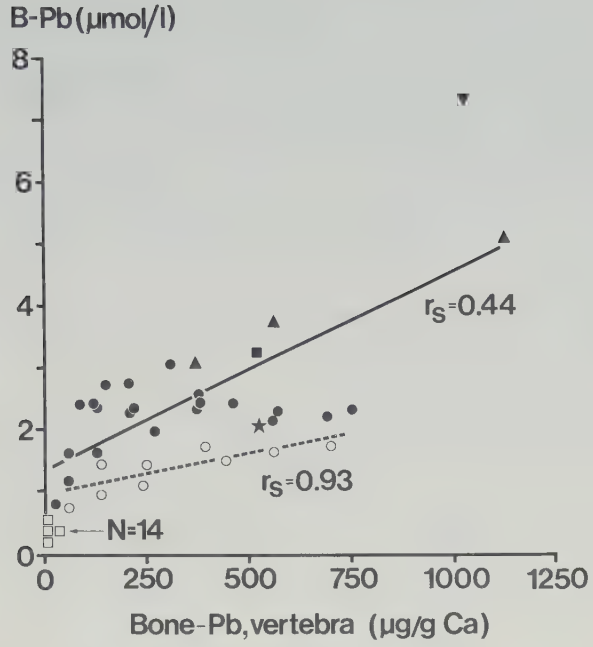

Fig. 4. Lead levels in vertebral bone (Bone-Pb) and blood (B-Pb) in 27 active and nine retired lead workers and in two referent subjects. Symbols as in fig. 2. Linear regression lines for active (full line, Y=0.0031X+1.4) and retired (dotted line, Y=0.0013X+0.9) workers are indicated.

DISCUSSION

Only about 25 per cent by weight of the vertebra is bone,[19] the rest is mainly bone marrow, which contains much lower lead levels than bone.[3] Thus, the lead level in the bone proper was probably about 4 times higher than the levels in the fresh biopsy samples. The impact of varying mass relations of bone/marrow between different biopsies was eliminated, at least to a great extent, by expressing the lead concentration in relation to calcium content.

The bone-Pb levels in the reference subjects were higher than in prehistoric Peruvians,[20] in accordance with studies of contemporary Scandinavians,[21,22] but considerably lower than in Germans,[13,15] Englishmen,[9] and Americans.[12,14,23]

The present bone-Pb concentrations in lead workers are in general accordance with those reported earlier in lead-exposed subjects.[2-8,10,12,13,15,22,24] Cases of clinical lead poisoning have displayed similar or higher levels.[13,23,25]

There was a rise of bone-Pb in vertebra with increasing exposure time. This is in agreement with earlier observations in lead workers[4,7,12] and in uranium miners, who accumulate lead-210 in the skeleton.[26-30] However, the variation of bone-Pb at a particular exposure time was considerable, which most probably was due

to differences in exposure intensity (absorbed lead), and inter-individual variations in lead metabolism.[3,31,32] Two active lead workers displayed a particularily rapid rise. Both had been intensely exposed, and had been hospitalized because of lead poisoning. On the other hand, two out of three active workers who had accumulated least lead in relation to time of employement, had been only slightly exposed.

To allow for some of the variations in exposure intensity and metabolism, the association between time-integrated B-Pb and vertebra bone-Pb was studied. The variation was less as compared to when only exposure time was employed, but it was still considerable.

The vertebra bone-Pb in the retired lead workers was significantly increased, which is consistent with earlier findings in lead workers[4-7,10,12,15,24] and in uranium miners.[26-28,33-34]

There was a general association between lead levels in finger-bone and vertebra. The finger bone-Pb was higher in the retired workers as compared to the active ones, which is most likely due to an almost three times longer exposure time and probably also a higher average exposure intensity. In contrast, there was no significant difference in vertebra bone-Pb between the groups. Further, while in the active workers there was no systematic difference between bone-Pb in finger and in vertebra the retired ones had systematically higher levels in finger which may indicate a faster elimination from vertebra than from finger-bone. However, some other possible explanations of this discrepancy have to be considered.

Firstly, there might be an effect of osteoporosis on vertebral bone-Pb.[35,36] However, since there was no correlation between age and calcium concentration in the biopsies, this impact should be limited. Secondly, in the retired workers, the biopsies were generally obtained after (average 1.4 yr) the finger measurements, during which time the vertebra bone-Pb had decreased and thus gives a spuriously high ratio between finger bone-Pb and vertebra bone-Pb. However, if the decrease rate were the same in finger and in vertebra, and the ratio was about 1.3 just prior to retirement, then the half-time for the elimination of lead has to be about 1 yr to give a ratio of about 4.0 after 2.6 yr of retirement. But, this is considerably shorter than earlier found in finger-bone (7 yr),[5,11] and presupposes unlikely high bone lead levels at the time of retirement. Instead, it seems, that after end of the exposure, the vertebra bone-Pb decreased faster than the finger bone-Pb.

Thus it may be assumed that the trabecular (cancellous or spongy) bone of vertebra represents a lead pool with a faster turn-over while the cortical (compact or dense) bone of the finger phalanx represents a slower one. This is in accordance with different turn-over rates reported for the two types of bone tissue.[19,37]

Assuming an average half-time of 7 yr for lead in finger-bone,[5,11] a ratio of 1.3 between levels in finger and vertebra at the end of exposure (corresponding to the ratio found in active workers), and a ratio of 4.0 after 2.6 yr of retirement, the median half-time of lead in vertebra in the retired workers would be 1.5 yr. A calculation of a rough half-time from the present data on accumulation of vertebra bone-Pb on time of employment in active workers would give a similar estimate (1.8 yr). It should be noted, though, that, the former figure denotes an effective half-time, which includes elimination and incorporation of lead in the

skeleton, while the latter only takes into account the elimination. The figures are thus not fully comparable. However, they indicate the approximate turn-over rate in vertebra.

On basis on the patterns of accumulation and elimination, it thus seems reasonable to claim the existence of at least two different lead pools in the skeleton. Moreover, this is in accordance with the difference in lead accumulation pattern on age in different bones in subjects without occupational exposure,[38] as well as with data on incorporation of lead in bone in animals,[39] and incorporation[40] and elimination[15] in man.

The lack of good agreement between lead levels in vertebra and finger in individual active workers, can thus be explained by different metabolic rates, the concentration in vertebra being more dependent upon recent exposure than the level in finger-bone, which mainly reflects the long-term accumulation.

An unlikely high finger bone-Pb of 40 µg/g (vertebra bone-Pb 3 µg/g) was measured in one of the active workers with the shortest exposure time (0.6 yr), his finger/vertebra ratio was 13. The next highest ratio in the active workers was 2.5. The former value may be due to finger skin contamination. There is, however, no reason to believe that contamination caused a general substancial elevation of finger bone-Pb, since, in an earlier study, no significant change was observed in a group of active workers when finger bone-Pb was measured just before as well as at the end of an exposure-free period averaging 5.3 months.

The turnover rate of lead in vertebra indicated by the present data is considerably faster than earlier estimates of the skeletal lead metabolism rate,[3,5,11,30,32,40,41] but is slower than a couple of observations on the decrease of lead level in trabecular bone in poisoned workers,[12,13] though one[12] was treated by chelating agents. Further, the turnover rate for lead seems to be faster than for two other "bone-seekers": strontium[37] and fluoride.[42] Moreover, recent data indicate that it is also different from that of calcium.[43] This pattern might be explained by a turnover of skeletal lead which is not only governed by bone remodeling, but also by a considerable exchange with the fraction of the skeleton which is not at that particular time engaged in remodeling.

The association between B-Pb and vertebra bone-Pb in retired lead workers suggests a release of lead from the skeleton into the blood stream. This is in accordance with earlier findings in studies of finger-bone in lead workers,[3-5] and various bones in uranium miners.[27-29] Such a release may cause a considerable impact (as much as 1 µmol/l) on B-Pb. The release of lead from the skeleton[44,45] causes a continous "endogenous" lead exposure, e.g. of the nervous system, for the rest of the worker´s life. The higher B-Pb at a particular bone-Pb in active as compared to retired workers is due to the difference in recent absorption.

The skeletal lead pool may constitute a risk, as osteolytic bone tumours may cause a rapid mobilization of lead,[27] perhaps even "endogenous" lead poisoning.[7,46]

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CHELATABLE LEAD VS. LEAD IN HUMAN TRABECULAR AND COMPACT BONE

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ABSTRACT

In active and retired lead workers, there was a close correlation between urinary excretion of lead during 6 h after intake of a single oral dose of 0.5 g penicillamine, and the excretion during 24 h. In chelation tests it is thus sufficient to collect urine for a few hours. There was a close correlation between the amount of chelatable lead on the one hand, and the blood-lead level as well as the lead level in biopsies of trabecular bone from vertebrae on the other, but there was no association with lead in compact bone, as measured in finger-bone by *in vivo* X-ray fluorescence. In conclusion, the chelatable lead probably mainly reflects the soft tissue lead pool and a fraction of the *trabecular* bone lead pool, which has a relatively rapid turnover. In contrast, it is not a valid indicator of the pool of lead, which has slowly accumulated in the *compact* bone, and it is thus not useful as a time-integrated index of the exposure over a long time.

INTRODUCTION

Lead accumulates in the skeleton, where it has a slow turnover, and - in lead workers - may amount to as much as 99% of the body burden (Skerfving et al 1983, 1985, and in press; Skerfving, in press). The skeletal lead level (Bone-Pb) reflects earlier lead absorption (Christoffersson et al 1984; Schütz et al, in press a and b), which makes it useful as a time-integrated exposure-index. Also, the skeletal lead may considerably affect the blood lead level (B-Pb; Christoffersson et al 1984; Schütz et al, in press a and b).

It is thus of interest to know the Bone-Pb in living individuals. It is possible to achieve this by chemical analysis of samples obtained by bone biopsy from the iliac crest (Westerman et al 1965) or the spinal process of a vertebra (Schütz et al, in press a). But this is, of course, a method which is not suited for routine use. More promising, from this point of view, is lead determination in finger-bone (Ahlgren et al 1976 and 1980; Christoffersson et al 1984 and in press) or tibia (Somervaille et al 1983; Wielopolski et al 1986) *in vivo* by X-ray fluorescence (XRF). However, the analysis requires fairly extensive equipment, which will not be readily available everywhere.

Lead mobilization tests, in which chelating agents are administered, and the resulting excretion of lead in the urine is measured, have been extensively employed for estimation of lead accumulation (cf. Skerfving, in press), and might thus be a valuable tool to determine the skeletal lead content. It has been reported that the chelatable lead is high, even many years after end of occupational exposure (Prerovska and Teisinger 1970), which may indicate an association with skeletal lead, which decreases only slowly after end of exposure (Christoffersson et al 1984 and in press; Schütz et al, in press a).

Further, a recent report claimed an association in lead workers between chelatable lead and lead content of the tibia, as determined by *in vivo* XRF (Wielopolski et al 1986). However, the skeletal lead pool is not homogenous (cf. Skerfving, in press). Data on one part of the skeleton may thus not be relevant to the total skeletal lead pool.

We here report data on the relationship between chelatable lead and Bone-Pb in finger-bone and vertebra in active and retired lead workers.

SUBJECTS STUDIED

Active lead workers

Twenty-one male active lead workers were studied. Twenty were working in a secondary smelter, one was a demolition worker. Their median age was 42 (range 19-65) yr and their median exposure time 7.5 (range 0.1-38) yr.

Retired lead workers

Fourteen male retired lead workers were studied. Thirteen had worked in the secondary smelter, the remaing one in a brass foundry. Their median age was 67 (range 46-71) yr and their median exposure time 28.5 (range 4.5-46) yr. A median time of 2.9 (range 1.0-5.6) yr had elapsed since end of expoure.

Non-exposed subjects

Two healthy males without occupational lead exposure were studied. Their age was 36 and 41 yr, respectively.

METHODS

Blood -lead level

Venous blood was obtained immediately before PCA ingestion from the cubital vein in metal-free heparinized tubes. Five ml samples were wet-ashed and lead was complexed with dithizone, extracted, and determined by flame atomic absorption spectrometry (AAS; Schütz and Skerfving 1976, Schütz 1979, Schütz et al 1984). The detection limit was 0.05 µmol/l (10 µg/l).

Each analytical series contained six samples, two blanks containing reagents only, and four "normal" blood samples, two with standard lead addition. Each sample was analysed in duplicate, in different series. The coefficient of variation, as calculated from duplicate analyses of 57 samples containing 0.5 µmol/l or less was 6.6%, in 25 samples containing 0.5-1 µmol/l 3.9%, in 58 samples containing 1-2 µmol/l 2.5%, and in 60 samples containing 2-3.5 µmol/l 2.0%.

The accuracy was tested twice each year in an inter-Nordic laboratory calibration program with 6-19 (mean 12) laboratories participating at each occasion. The regression function of our results (Y, µmol/l) on the average result of the other laboratories (X) was Y=1.008-0.052. Our results in the 115 samples (range 0.2-5.6 µmol/l) averaged 96.3% (range 80-112, 62% within 95-105%) of the mean of the other laboratories. Further, we participated in the U.K. External Quality Assessment Scheme in 1983-4. In the 38 samples analyzed, our results averaged 99% (range 90-120%, 74% within 95-105%) of the mean (2.42 µmol/l) of about 90 participating laboratories. Neither quality control series displayed any time trend.

Urinary lead level

Urine was collected in acid-washed polyethylene bottles. The volume and creatinine content was determined.

Lead levels in urine (U-Pb) in samples were analysed in duplicate in 5-20 ml samples (depending upon the expected concentration) by the method also used for B-Pb. The detection limit was 0.01 µmol/l. The precision, as calculated from duplicate analyses, was 5.3% in the range 0.05-0.5 µmol/l (N=48), 3.5% in the range 0.5-1.0 µmol/l (N=51), 3.3% in the range 1.0-2.0 µmol/l (N=17), and 2.7% above 2.0 µmol/l (N=11).

Creatinine levels in urine were determined according to a modified kinetic Jaffé method (Lustgarten and Wenk 1972).The method error at 10 consecutive analyses of one sample was 2%.

Lead mobilization test

The lead mobilization test was performed according to Ohlsson (1963), with some modifications. After at least two hours fasting, the subject emptied his urinary bladder in a bottle and he ingested 0.5 g of d-penicillamine (PCA; Cuprimine^{R}). All urine was collected for 6 h in another bottle, and then, separately, for an additional 18 h, to obtain the excretion during 24 h.

Skeletal lead level

Bone-Pb in *finger-bone* was analysed *in vivo* by an X-ray fluorescence (XRF) method, using ^{57}Co as the radiation source (Ahlgren et al 1976 and 1980; Christoffersson et al 1984 and in press). The detection limit was 20 µg/g (three standard deviations above the background). The accuracy was good when the results were compared with AAS (Christoffersson et al 1984). The precision of the method is about 15 %. In some cases, the measurements were not made simultaneously with the PCA test (from 2.5 yr before to 3.1 yr after). As a median for all subjects, the XRF measurements were made 0.1 yr before the chelation test.

In addition, lead levels were analysed in bone biopsies obtained from the spinous process of a lumbar *vertebra* (Schütz et al, in press a). Analysis of lead was performed by electrothermal AAS after combined dry and wet ashing. The detection limit was about 1 µg/g and the precision about 5%. Lead content was related to calcium (Ca) content, as determined by flame AAS. In some cases, the measurements were not made simultaneously with the PCA test (from 0.4 yr before to 0.9 yr after, median 0).

The project was approved by the Ethical Committee of Lund University. All subjects gave written consent.

Statistics

Almost only non-parametric statistics (Mann-Whitney U-test, Wilcoxon matched-pairs signed-ranks test, Spearman´s rank correlation test, r_S) was employed. All p-values are two-tailed. "Statistically significant" denotes $p \leq 0.05$.

RESULTS

In the total lead-worker population, the U-Pb was 0.016 µmol/mmol creatinine before chelation. There was no significant difference between active and retired workers (medians 0.023 *vs* . 0.014 µmol/mmol creatinine, $p>0.05$). The U-Pb among all lead workers was, of course, higher during 6 h (0.067 µmol/mmol creatinine; $p<0.0001$) and 24 h (0.041 µmol/mmol creatinine; $p<0.0001$), after ingestion of the chelating agent.

In the total lead-worker population, the excretion during 24 h after the PCA intake was twice as high as during 6 h (0.54 *vs* . 0.27 µmol, $N=33$, $p<0.001$). There was a close correlation between the excretion during these two time intervals (Figure 1; $r_S=0.98$, $p<0.02$). Thus, in the following, only the 6 h figures will be used.

The median excretion of lead after chelation in the active lead workers was about twice as high as in the retired ones (medians 0.37 *vs* . 0.17 µmol, $p=0.04$, table 1). All lead workers, active as well as retired ones, had higher levels than the two non-exposed workers.

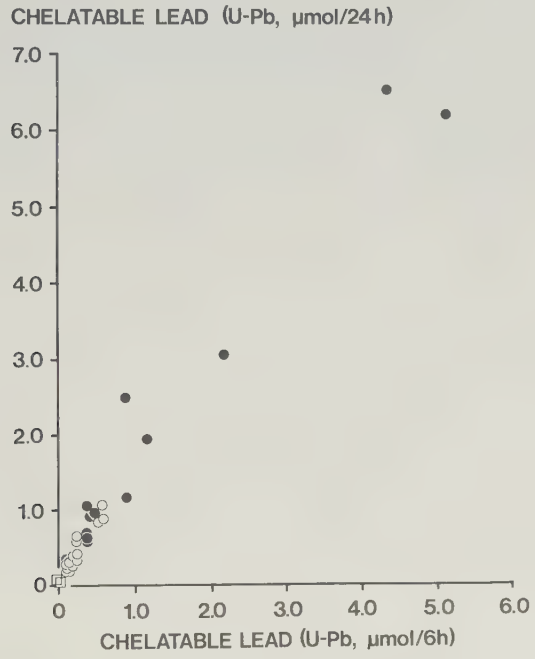


Fig. 1. Urinary excretion of lead 6 and 24 h after peroral penicillamine ingestion (chelatable lead) in 19 active (closed circles) and 14 retired (open circles) lead workers and two non-exposed subjects (open squares).

Table 1. Lead excretion in urine before (**U-Pb(pre-PCA)**), during the first six h (**U-Pb(PCA,6h)**), and during 24 h (**U-Pb(PCA,24h)**) after ingestion of penicillamine (PCA), and levels of lead in blood (**B-Pb**) and in bone (**Bone-Pb**) in active and in retired lead workers and in occupationally unexposed subjects. A significant difference between active and retired workers is indicated by *. Ranges are indicated within brackets.

Parameter	Lead workers		Retired		Non-exposed	
	N	Median	N	Median	N	Median
U-Pb (pre-PCA) µmol/mmol creat.	20	0.023 (0.002-0.156)	14	0.014 (0.004-0.048)	2	0.002-0.003
U-Pb (PCA,6h) µmol/mmol creat.	21	0.085 (0.003-1.225)	14	0.049 (0.012-0.116)	2	0.004
µmol	21	0.37	14	0.17*	2	0.01-0.02
U-Pb (PCA,24h) µmol/mmol creat.	21	0.042 (0.002-0.552)	14	0.029 (0.007-0.062)	2	0.002
µmol	19	0.61 (0.14-6.54)	14	0.32* (0.10-1.02)	2	0.04
B-Pb, µmol/l	21	2.20 (077-3.96)	14	1.49* (0.72.1.91)	2	0.19-0.22
Bone-Pb vertebra, µg/g Ca	11	150 (30-560)	10	340 (60-1170)	2	10-40
finger, µg/g	16	32 (<20-79)	14	72* (<20-135)	2	<20

The active workers had higher B-Pb than the retired ones (2.20 *vs* . 1.49 µmol/l, p=0.03). There was a close correlation between B-Pb and chelatable lead in the total material (Figure 2; N=37, r_S=0.93, p<0.02), as well as in the active workers (r_S=0.93, p<0.02), and in the retired ones (r_S=0.85, p<0.02). This applied also, when only those subjects were analysed, in whom skeletal lead levels had been determined (N=32, r_S=0.93, p<0.02). The linear correlation was closer when the logarithms of chelatable lead were employed (r=0.84), than when the original values were used (r=0.45).

The retired workers had numerically higher Bone-Pb in vertebra than the active ones (340 *vs* . 150 µg/g Ca), but the difference was not statistically significant (p>0.05). There was a close correlation between lead levels in vertebral bone and chelatable lead, in the total material (Figure 3; N=23, r_S=0.81, p<0.02), as well as in the active workers (r_S=0.91, p<0.02), and the retired ones (r_S=0.97, p<0.02). This was the case even when only Bone-Pb determinations made within 0.5 yr from the PCA test were employed (N=21, r_S=0.81, p<0.02). The active workers had significantly (p<0.002) higher ratios between chelatable lead and vertebral Bone-Pb than

The retired workers had higher Bone-Pb in finger than the active ones (72 *vs* . 32 µg/g, $p<0.02$). There was no correlation between Bone-Pb in finger and chelatable lead in the total material (Figure 4; N=32, $r_S=-0.21$, $p>0.05$), neither in the active workers ($r_S=-0.16$, $p>0.05$), nor in retired ones ($r_S=0.43$, $p>0.05$). This was the case even when only Bone-Pb determinations made within 0.5 yr from the PCA test were employed (N=17, $r_S=-0.32$, $p>0.05$). The active workers had significantly ($p<0.002$) higher ratios between chelatable lead and Bone-Pb in finger than had the retired ones.

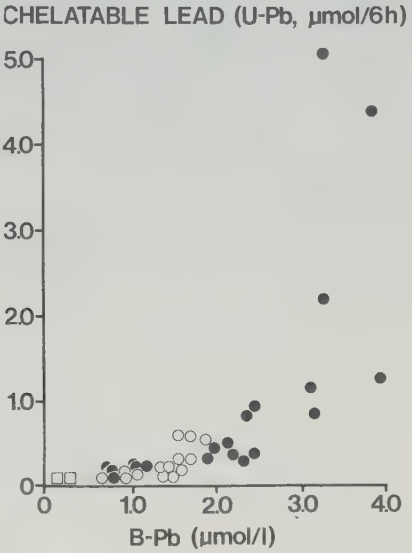
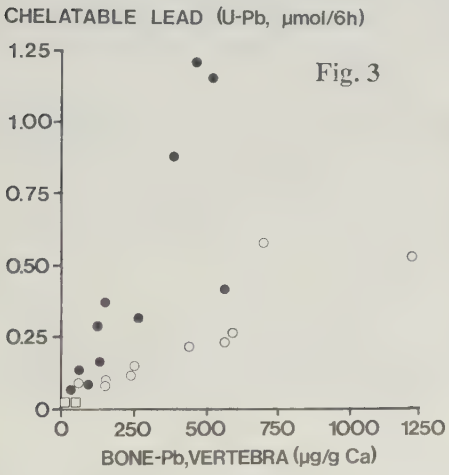


Fig. 2. Lead levels in blood (B-Pb) and urinary excretion of lead 6 h after peroral penicillamine ingestion (Chelatable lead) in 21 active (closed circles) and 14 retired (open circles) lead workers and in two non-exposed subjects (open squares).



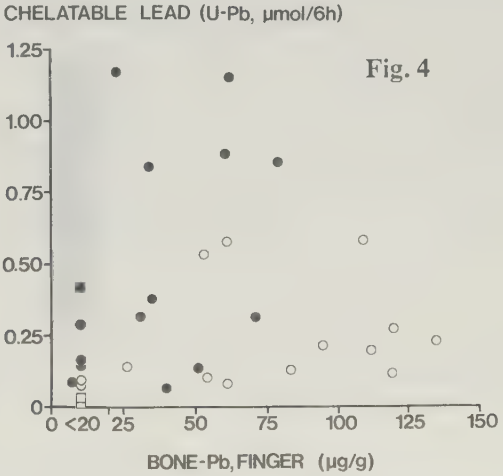


Fig. 3. Lead levels (Bone-Pb) in vertebral bone and urinary excretion of lead 6 h after peroral penicillamine ingestion (Chelatable lead) in 11 active (closed symbols) and 10 retired (open symbols) lead workers and in two non-exposed subjects (open squares).

Fig. 4. Lead levels (Bone-Pb) in finger-bone and urinary excretion of lead 6 h after peroral penicillamine ingestion (Chelatable lead) in 16 active (closed circles) and 14 retired (open circles) lead workers and in two non-exposed subjects (open squares). The area below the detection limit for Bone-Pb is shadowed.

DISCUSSION

The close correlation between the 6 and 24 h urinary lead excretion after administration of the chelating agent, is in agreement with earlier findings (Markowitz and Rosen 1984). It is thus sufficient to collect urine for a few hours, which facilitates the test considerably, and which also limits the risk of containation and incomplete sampling, which is always imminent at prolonged sampling time in persons not confined to a metabolic ward.

We employed penicillamine as the chelating agent, as it may be given orally, and thus renders a simple possibility for biological monitoring. Parenteral administration of calcium disodium edetate is the method mostly used in chelation tests (e.g. Brangstrup Hansen et al 1981; Markowitz and Rosen 1984; Wielopolski et al 1986). There is, however, no reason to believe that the lack of association between chelatable lead and Bone-Pb in finger was due to the choice of chelating agent.

The correlation between chelatable lead and B-Pb, especially in the active workers, strongly suggests that the chelatable lead reflects the soft tissue pool. The exponential relationship is in accordance with the B-Pb/U-Pb relationship in subjects, who had not taken chelate (Schütz and Skerfving 1976). It might be due to a relatively higher level of for PCA accessible lead in plasma (and probably also in the extracellular fluid) at high B-Pb (cf. Skerfving, in press).

The lead substitutes calcium in the skeleton. The skeleton contains more than one pool of lead (e.g. Skerfving et al 1985; Skerfving, in press). These various pools have different turnover rates (Rabinowitz et al 1977; Schütz et al, in press).

The vertebra contains mainly trabecular (cancellous or spongy) bone. This kind of bone contains a large fraction of bone marrow. However, marrow has a much lower lead concentration than the bone proper (Skerfving et al 1983). There was a correlation between chelatable lead and the Bone-Pb in vertebra. This is presumably due to a superficial fraction of the lead, which has a relatively rapid turnover, and thus is in equilibrium with the soft tissue pool. This is also in accordance with the close association between the rather rapidly metabolised lead in trabecular bone and the soft tissue pool (Schütz et al, in press a).

The higher level of cheletable lead, at a certain vertebral lead content, in active workers, as compared to retired ones, is probably due to a larger soft tissue pool in the former, as a result of higher recent absorption. Also, they may have had lead in the gastrointestinal tract, which may have be- come chelated, absorbed, and excreted. Although there is a correlation on group basis, the variation of the size of the soft tissue pool with recent exposure makes cheletable lead a poor index of the lead content in trabecular bone.

The finger-bone contains a considerable fraction of cortical (compact or dense) bone. Finger bone has a rather slow turnover (Christoffersson et al, in press). It is thus not surprising that there was no association with chelatable lead. The lack of association is supported by the fact that retired workers had higher Bone-Pb levels in finger (probably due to longer and more intense exposure), but lower amounts of chelatable lead (due to lower recent exposure), than the active ones.

This absence of correlation may seem to be in contrast with Wielopolski et al (1986), who showed that there was an association between Bone-Pb as measured by XRF in the tibia in active workers and 48 h urinary lead excretion following intravenous administration of 1 g calcium disodium edetate. However, beside the difference as regards the chelation test and the part of the skeleton which was analysed, there is one more important difference. Wielopolski et

al (1986) measured Pb L X-rays, which mainly originate from lead contained in the surface layer of the bone, while we employed Pb K X-rays, which are, to a great extent, relevant to all lead contained in the bone, in the deep bulk parts in addition to the surface. This may well explain the difference.

It is known that there is a small calcium pool in the skeleton, which has a rapid turnover, and which rapidly equilibrates with the soft tissue calcium content (Bauer et al 1955). It is probable that this pool is partly located on the surface of the bone (including the trabecular bone). It probably rapidly equilibrates with the soft tissue lead pool (Heard and Chamberlain 1984). It is thus reasonable to assume, that measurement of both lead on the bone surface, by use of L X-rays, and of chelatable lead, mainly reflects a rather rapid pool, which, to a great part, reflects the recent absorption.

But the chelatable lead thus does not usually reflect the much larger amount of lead, which has slowly diffused so deep into the compact bulk parts of the skeleton, that it is not readily available to the chelating agent. That pool, which does reflect the time-integrated lead absorption during at least one decade (Christoffersson et al 1984; Schütz et al, in press a), may thus not be estimated by a chelatable lead test. But it can be measured by use of *in vivo* XRF, provided that K X-rays are employed.

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